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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No.	BB-1037-F
First Named Inventor or Application Identifier	Saverio Carl Falco
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APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

ADDRESS TO: **Assistant Commissioner for Patents**
Box Patent Application
Washington, DC 20231

1. [X] Fee (Authority to charge deposit account below.) *(Submit an original, and a duplicate for fee processing)*
2. [X] Specification [Total Pages 206] *(preferred arrangement set forth below)*
   - Descriptive title of the invention
   - Cross References to Related Applications *(if needed)*
   - Statement Regarding Fed sponsored R & D *(if needed)*
   - Reference to Microfiche Appendix *(if filed)*
   - Background of the Invention
   - Brief Summary of the Invention
   - Brief Description of the Drawings *(if filed)*
   - Detailed Description
   - Claim(s)
   - Abstract of the Disclosure
3. [X] Drawing(s) *(35 USC 113)* [Total Sheets 25]
4. [ ] Oath or Declaration [Total Pages 0]
   a. [ ] Newly executed (original or copy)
   b. [ ] Copy from a prior application (37 CFR 1.63(d)) *(for continuation/divisional with Box 14 completed)*
      i. [ ] DELETION OF INVENTORS Signed Statement below at 15 deleting inventor(s) named in the prior application, see 37 CFR 1.63(d)(2) and 1.33(b).
5. [ ] Incorporation by Reference *(useable if Box 4b is checked)* The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.
6. [ ] Microfiche Computer Program (Appendix)
7. Nucleotide and/or Amino Acid Sequence Submission *(if applicable, all necessary)*
   a. [ ] Computer Readable Copy
   b. [X] Paper Copy
   c. [ ] Statement verifying identity of above copies

ACCOMPANYING APPLICATION PARTS

8. [X] Power of Attorney
9. [ ] Information Disclosure Statement (IDS)/Cover Letter plus PTO-1449 [ ] Copies of IDS Citations
10. [ ] Preliminary Amendment
11. [X] Return Receipt Postcard (MPEP 503) *(Should be specifically itemized)*
12. [ ] Certified Copy of Priority Document(s) *(if foreign priority is claimed)*
13. [X] Other: Unsigned Declaration

14. **If a CONTINUING APPLICATION**, *check appropriate box and supply the requisite information:*
    [ ] Continuation [ ] Divisional [X] Continuation-in-part (CIP) of prior Application No.: 08/824,627

15. [ ] **DELETION OF INVENTOR(S) STATEMENT**: This application is being filed by less than all the inventors named in the prior application. In accordance with 37 CFR 1.63(d)(2) and 1.33(b), the Assistant Commissioner is requested to delete the name(s) of the following person or persons who are not inventors of the invention being claimed in this application:

16. [X] Amend the specification by inserting before the first line the sentence:

-- This is a continuation-in-part of Application No. 08/824,627 filed March 27, 1997, pending, which is a continuation-in-part of Application No. 08/474,633, filed on June 7, 1995, pending, which is a continuation-in-part of Application No. 08/178,212, filed on January 6, 1994 which was the national filing of PCT/US93/02480, now abandoned, filed on March 18, 1993 and which is a continuation-in-part of Application No. 07/855,414, filed on March 19, 1992, now abandoned. now pending. --

17. [ ] Cancel in this application original claims ___ of the prior application before calculating the filing. (At least one original independent claim must be retained for filing purposes.)
18. [ ] Priority of foreign Application No. _____ filed on _____ in _____ (country) is claimed under 35 U.S.C. 119.

CLAIMS	(1) FOR	(2) NUMBER FILED	(3) NUMBER EXTRA	(4) RATE	(5) CALCULATIONS
	TOTAL CLAIMS (37 CFR 1.16(c))	**30 - 20 =**	**10**	**x $ 22 =**	**$ 220.00**
	INDEPENDENT CLAIMS (37 CFR 1.16(b))	**3 - 3 =**	**0**	**x $ 82 =**	**0**
	MULTIPLE DEPENDENT CLAIM(S) (if applicable)			**+ $ 270 =**	**270.00**
				BASIC FEE (37 CFR 1.16(a))	**+ $ 790.00**
				TOTAL =	**$ 1280.00**

19. The Commissioner is hereby authorized to credit overpayments or charge the following fees to Deposit Account No. 04-1928:

a. [X] Fees required under 37 CFR 1.16.

b. [X] Fees required under 37 CFR 1.17.

20. [ ] Other:

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NAME	Lynne M. Christenbury	REG. NO.: 30,971
SIGNATURE	Lynne M. Christenbury	
DATE	March 27, 1998	

TITLE

CHIMERIC GENES AND METHODS FOR INCREASING THE LYSINE CONTENT OF THE SEEDS OF PLANTS

CROSS-REFERENCE TO RELATED APPLICATIONS

This is a continuation-in-part of Serial No.08/824,627, filed on March 27, 1997, pending, which is a continuation-in-part of Serial No. 08/474,633, filed on June 7, 1995, pending, which is a continuation-in-part of Serial No. 08/178,212, filed on January 6, 1994 which was the national filing of PCT/US93/02480, now abandoned, filed on March 18, 1993 and which is a continuation-in-part of Serial No. 07/855,414, filed on March 19, 1992, now abandoned.

FIELD OF THE INVENTION

This invention relates to chimeric genes and methods for increasing the lysine content of the seeds of plants and, in particular, to two chimeric genes, a first encoding plant lysine ketoglutarate reductase (LKR) and a second encoding lysine-insensitive dihydrodipicolinic acid synthase (DHDPS) which is operably linked to a plant chloroplast transit sequence, all operably linked to plant seed-specific regulatory sequences.

BACKGROUND OF THE INVENTION

Many vertebrates, including man, lack the ability to manufacture a number of amino acids and, therefore, require these amino acids preformed in the diet. These are called essential amino acids. Human food and animal feed derived from many grains are deficient in some of the ten essential amino acids. In corn (*Zea mays L.*), lysine is the most limiting amino acid for the dietary requirements of many animals. Soybean (*Glycine max L.*) meal is used as an additive to corn based animal feeds primarily as a lysine supplement. Thus, an increase in the lysine content of either corn or soybean would reduce or eliminate the need to supplement mixed grain feeds with lysine produced via fermentation of microbes.

Plant breeders have long been interested in using naturally occurring variations to improve protein quality and quantity in crop plants. Maize lines containing higher than normal levels of lysine (70%) have been identified [Mertz et al. (1964) *Science 145*:279, Mertz et al. (1965) *Science 150*:1469-70]. However, these lines which incorporate a mutant gene, opaque-2, exhibit poor agronomic qualities (increased susceptibility to disease and pests, 8-14% reduction in yield, low kernel weight, slower drying, lower dry milling yield of flaking grits, and increased storage problems) and thus are not commercially useful [Deutscher (1978) *Adv. Exp. Medicine and Biology 105*:281-300]. Quality Protein Maize (QPM) bred at CIMMYT using the opaque-2 and sugary-2 genes and associated modifiers has a hard endosperm and enriched levels of lysine and

tryptophan in the kernels [Vasal, S. K., et al. *Proceedings of the 3rd seed protein symposium*, Gatersleben, August 31 - September 2, 1983]. However, the gene pools represented in the QPM lines are tropical and subtropical. Quality Protein Maize is a genetically complex trait and the existing lines are not easily adapted to the dent germplasm in use in the United States, preventing the adoption of QPM by corn breeders.

The amino acid content of seeds is determined primarily (90-99%) by the amino acid composition of the proteins in the seed and to a lesser extent (1-10%) by the free amino acid pools. The quantity of total protein in seeds varies from about 10% of the dry weight in cereals to 20-40% of the dry weight of legumes. Much of the protein-bound amino acids is contained in the seed storage proteins which are synthesized during seed development and which serve as a major nutrient reserve following germination. In many seeds the storage proteins account for 50% or more of the total protein.

To improve the amino acid composition of seeds genetic engineering technology is being used to isolate, and express genes for storage proteins in transgenic plants. For example, a gene from Brazil nut for a seed 2S albumin composed of 26% sulfur-containing amino acids has been isolated [Altenbach et al. (1987) *Plant Mol. Biol. 8*:239-250] and expressed in the seeds of transformed tobacco under the control of the regulatory sequences from a bean phaseolin storage protein gene. The accumulation of the sulfur-rich protein in the tobacco seeds resulted in an up to 30% increase in the level of methionine in the seeds [Altenbach et al. (1989) *Plant Mol. Biol. 13*:513-522]. However, no plant seed storage proteins similarly enriched in lysine relative to average lysine content of plant proteins have been identified to date, preventing this approach from being used to increase lysine.

An alternative approach is to increase the production and accumulation of specific free amino acids such as lysine via genetic engineering technology. However, little guidance is available on the control of the biosynthesis and metabolism of lysine in the seeds of plants.

Lysine, along with threonine, methionine and isoleucine, are amino acids derived from aspartate, and regulation of the biosynthesis of each member of this family is interconnected. Regulation of the metabolic flow in the pathway appears to be primarily via end products. The first step in the pathway is the phosphorylation of aspartate by the enzyme aspartokinase (AK), and this enzyme has been found to be an important target for regulation in many organisms. However, detailed physiological studies on the flux of 4-carbon molecules through the aspartate pathway have been carried out in the model plant system

Lemna paucicostata [Giovanelli et al. (1989) *Plant Physiol. 90*:1584-1599]. It was stated in this reference that "These data now provide definitive evidence that the step catalyzed by aspartokinase is not normally an important site for regulation of the entry of 4-carbon units into the aspartate family of amino acids [in plants]."

The aspartate family pathway is also believed to be regulated at the branch-point reactions. For lysine this is the condensation of aspartyl β-semialdehyde with pyruvate catalyzed by dihydrodipicolinic acid synthase (DHDPS), while for threonine and methionine the reduction of aspartyl β-semialdehyde by homoserine dehydrogenase (HDH) followed by the phosphorylation of homoserine by homoserine kinase (HK) are important points of control.

The *E. coli dapA* gene encodes a DHDPS enzyme that is about 20-fold less sensitive to inhibition by lysine than a typical plant DHDPS enzyme, e.g., wheat germ DHDPS. The *E. coli dapA* gene has been linked to the 35S promoter of Cauliflower Mosaic Virus and a plant chloroplast transit sequence. The chimeric gene was introduced into tobacco cells via transformation and shown to cause a substantial increase in free lysine levels in leaves [Glassman et al. (1989) PCT Patent Appl. PCT/US89/01309, Shaul et al. (1992) *Plant Jour. 2*:203-209, Galili et al. (1992) EPO Patent Appl. 91119328.2]. However, the lysine content of the seeds was not increased in any of the transformed plants described in these studies. The same chimeric gene was also introduced into potato cells and lead to small increases in free lysine in leaves, roots and tubers of regenerated plants [Galili et al. (1992) EPO Patent Appl. 91119328.2, Perl et al. (1992) *Plant Mol. Biol. 19*:815-823]. These workers have also reported on the introduction of an *E. coli* lysC gene that encodes a lysine-insensitive AK enzyme into tobacco cells via transformation [Galili et al. (1992) Eur. Patent Appl. 91119328.2; Shaul et al. (1992) Plant Physiol. 100:1157-1163]. Expression of the *E. coli* enzyme results in increases in the levels of free threonine in the leaves and seeds of transformed plants. Crosses of plants expressing *E. coli* DHDPS and AK resulted in progeny that accumulated more free lysine in leaves than the parental DHDPS plant, but less free threonine in leaves than the parental AK plant. No evidence for increased levels of free lysine in seeds was presented.

The limited understanding of the details of the regulation of the biosynthetic pathway in plants makes the application of genetic engineering technology, particularly to seeds, uncertain. There is little information available on the source of the aspartate-derived amino acids in seeds. It is not known, for example, whether they are synthesized in seeds, or transported to the seeds from leaves, or both, from most plants. In addition, free amino acids make up only a small fraction of the total amino acid content of seeds. Therefore, over-accumulation of

free amino acids must be many-fold in order to significantly affect the total amino acid composition of the seeds. Furthermore, little is known about catabolism of free amino acids in seeds. Catabolism of free lysine has been observed in developing endosperm of corn and barley. The first step in the catabolism of lysine is believed to be catalyzed by lysine-ketoglutarate reductase (LKR) [Brochetto-Braga et al. (1992) *Plant Physiol. 98*:1139-1147]. This protein is actually a bifunctional enzyme that is also responsible for catalysis of the presumed second reaction in the catabolism of lysine, saccharopine dehydrogenase (SDH) [Goncalves-Butruille et al. (1996) Plant Physiol. 110:765-771]. There are only a few reports of the isolation of genomic or cDNA clones encoding various portions of LKR/SDH proteins from plants. GenBank accession ATU9579 presents the sequence of a full-length cDNA clone for the bifunctional enzyme from *Arabidopsis thaliana*. The protein encoded by this clone is a homologue of both LKR and SDH proteins from fungal organisms. The DNA sequence for the genomic clone from *Arabidopsis* is also available as GenBank accession U95758 (Tang, et al. (1997) Plant Cell 9:1305-1316 and Epelbaum, et al. (1997) Plant Mol. Biol. 35: 735-748). GenBank accession AF003551 discloses a cDNA from corn which would direct the synthesis of a polypeptide from within the SDH domain of LKR/SDH proteins. GenBank accession AF042184 discloses the sequence of a cDNA from *Brassica napus* that is homologous to a relatively short portion of the full length clone from *Arabidodpsis*. However, whether such catabolic pathways are widespread in plants and whether they affect the level of accumulation of free amino acids is unknown. Finally, the effects of over-accumulation of a free amino acid such as lysine or threonine on seed development and viability is not known.

Heretofore, no method to increase the level of lysine in seeds via genetic engineering was known. Thus, there is a need for genes, chimeric genes, and methods for expressing them in seeds so that an over-accumulation of lysine in seeds will result in an improvement in nutritional quality.

SUMMARY OF THE INVENTION

This invention concerns an isolated nucleic acid fragment comprising a nucleic acid sequence encoding all or part of lysine ketoglutarate reductase.

In another embodiment this invention concerns a chimeric gene comprising the aforesaid nucleic acid fragment encoding all or part of lysine ketoglutarate reductase, or a subfragment thereof, operably linked to suitable seed-specific regulatory sequences wherein said chimeric gene reduces lysine ketoglutarate reductase activity in seeds of transformed plants, as well as a plant cell or plant seed transformed with the aforesaid chimeric gene..

In a third embodiment this invention concerns a plant cell wherein lysine ketoglutarate reductase activity is reduced due to a mutation in a gene encoding lysine ketoglutarate reductase.

In a fourth embodiment this invention concerns a plant seed wherein lysine ketoglutarate reductase activity is reduced due to a mutation in a gene encoding lysine ketoglutarate reductase.

In a fifth embodiment this invention concerns a method for reducing lysine ketoglutarate reductase activity in a plant seed which comprises:

(a) transforming plant cells with the chimeric gene comprising the aforesaid nucleic acid fragment encoding all or part of lysine ketoglutarate reductase or a subfragment thereof, operably linked to suitable seed-specific regulatory sequences wherein said chimeric gene reduces lysine ketoglutarate reductase activity in seeds of transformed plants;

(b) regenerating fertile mature plants from the transformed plant cells obtained from step (a) under conditions suitable to obtain seeds;

(c) screening progeny seed of step (b) for reduced lysine ketoglutarate reductase activity; and

(d) selecting those lines whose seeds contain for reduced lysine ketoglutarate reductase activity.

In a sixth embodiment this invention concerns a nucleic acid fragment comprising

(a) a first chimeric gene comprising the aforesaid nucleic acid fragment encoding all or part of lysine ketoglutarate or a subfragment thereof, operably linked to suitable seed-specific regulatory sequences wherein said chimeric gene reduces lysine ketoglutarate reductase activity in seeds of transformed plants and

(b) a second chimeric gene wherein a nucleic acid fragment encoding dihydrodipicolinic acid synthase which is insensitive to inhibition by lysine is operably linked to a plant chloroplast transit sequence and to a plant seed-specific regulatory sequence.

A seventh embodiment of this invention concerns a plant and a seed comprising in its genome the aforesaid nucleic acid fragments or the first and second aforesaid chimeric genes.

BRIEF DESCRIPTION OF THE DRAWINGS AND SEQUENCE DESCRIPTIONS

The invention can be more fully understood from the following detailed description and the accompanying drawings and the sequence descriptions which form a part of this application.

Figure 1 shows an alpha helix from the side and top views.

Figure 2 shows end (Figure 2a) and side (Figure 2b) views of an alpha helical coiled-coil structure.

Figure 3 shows the chemical structure of leucine and methionine emphasizing their similar shapes.

Figure 4a shows a schematic representation of a leaf gene expression cassette; Figure 4b shows a schematic representation of a seed-specific gene expression cassette.

Figure 5 shows a map of the binary plasmid vector pZS97K.

Figure 6 shows a map of the binary plasmid vector pZS97.

Figure 7A shows a map of the binary plasmid vector pZS199; Figure 7B shows a map of the binary plasmid vector pFS926; Figure 7C shows a map of the binary plasmid vector pBT593; Figure 7D shows a map of the binary plasmid vector pBT597.

Figure 8A shows a map of the plasmid vector pBT603; Figure 8B shows a map of the plasmid vector pBT614.

Figure 9 shows the amino acid sequence similarity between the polypeptides encoded by two plant cDNAs and fungal SDH (glutamate-forming).

Figure 10 depicts the strategy for creating a vector (pSK5) for use in construction and expression of the SSP gene sequences.

Figure 11 shows the strategy for inserting oligonucleotide sequences into the unique Ear I site of the base gene sequence.

Figure 12 shows the insertion of the base gene oligonucleotides into the Nco I/EcoR I sites of pSK5 to create the plasmid pSK6. This base gene sequence was used as in Figure 8 to insert the various SSP coding regions at the unique Ear I site to create the cloned segments listed.

Figure 13 shows the insertion of the 63 bp "segment" oligonucleotides used to create non-repetitive gene sequences for use in the duplication scheme in Figure 12.

Figure 14 (A and B) shows the strategy for multiplying non-repetitive gene "segments" utilizing in-frame fusions.

Figure 15 shows the vectors containing seed specific promoter and 3' sequence cassettes. SSP sequences were inserted into these vectors using the Nco I and Asp718 sites.

Figure 16 shows a map of the plasmid vector pML63.

Figure 17 shows a map of the plasmid vector pBT680.

Figure 18 shows a map of the plasmid vector pBT681.

Figure 19 shows a map of the plasmid vector pLH104.

Figure 20 shows a map of the plasmid vector pLH105.

Figure 21 shows a map of the plasmid vector pBT739.

Figure 22 shows a map of the plasmid vector pBT756.

SEQ ID NO:1 shows the nucleotide and amino acid sequence of the coding region of the wild type *E. coli* lysC gene, which encodes AKIII, described in Example 1.

SEQ ID NOS:2 and 3 were used in Example 2 to create an Nco I site at the translation start codon of the *E. coli* lysC gene.

SEQ ID NOS:4 and 5 were used in Example 3 as PCR primers for the isolation of the *Corynebacterium* dapA gene.

SEQ ID NO:6 shows the nucleotide and amino acid sequence of the coding region of the wild type *Corynebacterium* dapA gene, which encodes lysine-insensitive DHDPS, described in Example 3.

SEQ ID NO:7 was used in Example 4 to create an Nco I site at the translation start codon of the *E. coli* dapA gene.

SEQ ID NOS:8, 9, 10 and 11 were used in Example 6 to create a chloroplast transit sequence and link the sequence to the *E. coli* lysC, *E. coli* lysC-M4, *E. coli* dapA and *Corynebacteria* dapA genes.

SEQ ID NOS:12 and 13 were used in Example 6 to create a Kpn I site immediately following the translation stop codon of the *E. coli* dapA gene.

SEQ ID NOS:14 and 15 were used in Example 6 as PCR primers to create a chloroplast transit sequence and link the sequence to the *Corynebacterium* dapA gene.

SEQ ID NOS:16-92 represent nucleic acid fragments and the polypeptides they encode that are used to create chimeric genes for lysine-rich synthetic seed storage proteins suitable for expression in the seeds of plants.

SEQ ID NO:93 was used in Example 6 as a constitutive expression cassette for corn.

SEQ ID NOS:94-99 were used in Example 6 to create a corn chloroplast transit sequence and link the sequence to the *E. coli* lysC-M4 gene.

SEQ ID NOS:100 and 101 were used in Example 6 as PCR primers to create a corn chloroplast transit sequence and link the sequence to the *E. coli* dapA gene.

SEQ ID NOS:102 and 103 are cDNAs for plant lysine ketoglutarate reductase/saccharopine dehydrogenase from *Arabidopsis thaliana*.

SEQ ID NOS:104 and 105 are polypeptides homologous to fungal saccharopine dehydrogenase (glutamate-forming) encoded by SEQ ID NOS:102 and 103, respectively.

SEQ ID NOS:106 and 107 were used in Example 25 as PCR primers to add Nco I and Kpn I sites at the 5' and 3' ends of the corn DHDPS gene.

SEQ ID NOS:108 and 109 were used for PCR amplification of a 2.24 kb DNA fragment from genomic *Arabidopsis* DNA.

SEQ ID NO:110 shows the sequence of the *Arabidopsis* LKR/SDH genomic DNA fragment.

SEQ ID NO:111 shows the sequence of the *Arabidopsis* LKR/SDH cDNA.

SEQ ID NO:112 shows the deduced amino acid sequence of *Arabidopsis* LKR/SDH protein.

SEQ ID NOS:113 and 114 were used for PCR amplification of soybean and corn LKR/SDH cDNA fragment.

SEQ ID NO:115 shows the sequence of a soybean LKR/SDH cDNA fragment.

SEQ ID NO:116 shows the sequence of a corn LKR/SDH cDNA fragment.

SEQ ID NO:117 shows the deduced partial amino acid sequence of soybean LKR/SDH protein.

SEQ ID NO:118 shows the deduced partial amino acid sequence of corn LRK/SDH protein.

SEQ ID NO:119 shows the sequence of a 2582 nucleotide cDNA from soybean.

SEQ ID NO:120 shows the sequence of a 3265 nucleotide cDNA from corn.

SEQ ID NO:121 shows the deduced partial amino acid sequence of soybean LKR/SDH protein encoded by nucleotides 3 through 2357 of SEQ ID NO:119.

SEQ ID NO:122 shows the deduced partial amino acid sequence of soybean LKR/SDH protein encoded by nucleotides 3 through 3071 of SEQ ID NO:120.

SEQ ID NO:123 is a nucleotide sequence corresponding to nucleotides 1 through 1908 of SED ID NO:120.

SEQ ID NO:124 is the deduced amino acid sequence from SEQ ID NO:123.

SEQ ID NO:125 shows the sequence of a 720 nucleotide LKR/SDH cDNA from rice.

SEQ ID NO:126 shows the deduced partial amino acid sequence of rice LKR/SDH protein encoded by nucleotides 2 through 720 of SEQ ID NO:125.

SEQ ID NO:127 shows the sequence of a 308 nucleotide LKR/SDH cDNA from rice.

SEQ ID NO:128 shows the deduced partial amino acid sequence of rice LKR/SDH protein encoded by nucleotides 1 through 129 of SEQ ID NO:127.

SEQ ID NO:129 shows the sequence of a 429 nucleotide cDNA from wheat.

SEQ ID NO:130 shows the deduced partial amino acid sequence of wheat LKR/SDH protein encoded by nucleotides 1 through 252 of SEQ ID NO:129.

SEQ ID NO:131 shows the SDH coding region of the *Arabidopsis* cDNA clone.

SEQ ID NO:132 shows the amino acid sequence of the SDH domain of the *Arabidopsis* LKR/SDH protein.

The Sequence Descriptions contain the one letter code for nucleotide sequence characters and the three letter codes for amino acids as defined in conformity with the IUPAC-IUB standards described in Nucleic Acids Research 13:3021-3030(1985) and in the Biochemical Journal 219 (No. 2):345-373(1984) which are incorporated by reference herein.

DETAILED DESCRIPTION OF THE INVENTION

Nucleic acid fragments and procedures are described which are useful for increasing the accumulation of lysine in the seeds of transformed plants, as compared to levels of lysine in untransformed plants. In order to increase the accumulation of free lysine in the seeds of plants via genetic engineering, a determination was made of which enzymes in this pathway controlled the pathway in the seeds of plants. In order to accomplish this, genes encoding enzymes in the pathway were isolated from bacteria. In some cases, mutations in the genes were obtained so that the enzyme encoded was made insensitive to end-product inhibition. Intracellular localization sequences and suitable regulatory sequences for expression in the seeds of plants were linked to create chimeric genes. The chimeric genes were then introduced into plants via transformation and assessed for their ability to elicit accumulation of the lysine in seeds.

A unique first nucleic acid fragment is provided which comprises two nucleic acid subfragments (subsequences), one encoding LKR and the other encoding DHDPS which is substantially insensitive to feedback inhibition by lysine. For the purposes of the present application, the term substantially insensitive will mean at least 20-fold less sensitive to feedback inhibition by lysine than a typical plant enzyme catalyzing the same reaction. It has been found that a combination of subfragments successfully increases the lysine accumulated in seeds of transformed plants as compared to untransformed host plants.

It also has been discovered that the full potential for accumulation of excess free lysine in seeds is reduced by lysine catabolism. Furthermore, it has been discovered that lysine catabolism results in the accumulation of lysine breakdown

products such as saccharopine and α-amino adipic acid. Provided herein are two alternative routes to reduce the loss of excess lysine due to catabolism and to reduce the accumulation of lysine breakdown products. In the first approach, lysine catabolism is prevented through reduction in the activity of the enzyme lysine ketoglutarate reductase (LKR), which catalyzes the first step in lysine breakdown. This can be accomplished by introducing a mutation that reduces or eliminates enzyme function in the plant gene that encodes LKR. Such mutations can be identified in lysine over-producer lines by screening mutants for a failure to accumulate the lysine breakdown products, saccharopine and α-amino adipic acid. Alternatively, several procedures to isolate plant LKR genes are provided; nucleic acid fragments containing plant LKR cDNAs are also provided. Chimeric genes for expression of antisense LKR RNA or for cosuppression of LKR in the seeds of plants can then be created. The chimeric LKR gene is linked to the chimeric genes encoding lysine insensitive DHDPS and both are introduced into plants via transformation simultaneously, or the chimeric genes are brought together by crossing plants transformed independently with each of the chimeric genes.

In the second approach, excess free lysine is incorporated into a form that is insensitive to breakdown, e.g., by incorporating it into a di-, tri- or oligopeptide, or preferably a lysine-rich storage protein. The lysine-rich storage protein chosen should contain higher levels of lysine than average proteins. Ideally, these storage proteins should contain at least 15% lysine by weight. The design of a preferred class of polypeptides which can be expressed *in vivo* to serve as lysine-rich seed storage proteins is provided. Genes encoding the lysine-rich synthetic storage proteins (SSP) are synthesized and chimeric genes wherein the SSP genes are linked to suitable regulatory sequences for expression in the seeds of plants are created. The SSP chimeric gene is then linked to the chimeric DHDPS gene and both are introduced into plants via transformation simultaneously, or the genes are brought together by crossing plants transformed independently with each of the chimeric genes.

A method for transforming plants is taught herein wherein the resulting seeds of the plants have at least ten percent, preferably ten percent to four-fold greater, lysine than do the seeds of untransformed plants. Provided as examples herein are transformed rapeseed plants with seed lysine levels increased by 100% over untransformed plants and soybean plants with seed lysine levels increased by four-fold over lysine levels of untransformed plants, and corn plants with seed lysine levels increased by 130%.

In the context of this disclosure, a number of terms shall be utilized. As used herein, the term "nucleic acid" refers to a large molecule which can be single-stranded or double-stranded, composed of monomers (nucleotides) containing a sugar, phosphate and either a purine or pyrimidine. A "nucleic acid fragment" is a fraction of a given nucleic acid molecule. In higher plants, deoxyribonucleic acid (DNA) is the genetic material while ribonucleic acid (RNA) is involved in the transfer of the information in DNA into proteins. A "genome" is the entire body of genetic material contained in each cell of an organism. The term "nucleotide sequence" refers to a polymer of DNA or RNA which can be single- or double-stranded, optionally containing synthetic, non-natural or altered nucleotide bases capable of incorporation into DNA or RNA polymers.

As used herein, the term "homologous to" refers to the complementarity between the nucleotide sequence of two nucleic acid molecules or between the amino acid sequences of two protein molecules. Quantitative estimates of homology are provided by either DNA-DNA or DNA-RNA hybridization under conditions of stringency as is well understood by those skilled in the art [as described in Hames and Higgins (eds.) Nucleic Acid Hybridisation, IRL Press, Oxford, U.K.]; or by the comparison of sequence similarity between two nucleic acids or proteins.

As used herein, "essentially similar" refers to DNA sequences that may involve base changes that do not cause a change in the encoded amino acid, or which involve base changes which may alter one or more amino acids, but do not affect the functional properties of the protein encoded by the DNA sequence. It is therefore understood that the invention encompasses more than the specific exemplary sequences. Modifications to the sequence, such as deletions, insertions, or substitutions in the sequence which produce silent changes that do not substantially affect the functional properties of the resulting protein molecule are also contemplated. For example, alteration in the gene sequence which reflect the degeneracy of the genetic code, or which result in the production of a chemically equivalent amino acid at a given site, are contemplated; thus, a codon for the amino acid alanine, a hydrophobic amino acid, may be substituted by a codon encoding another less hydrophobic residue, such as glycine, or a more hydrophobic residue, such as valine, leucine, or isoleucine. Similarly, changes which result in substitution of one negatively charged residue for another, such as aspartic acid for glutamic acid, or one positively charged residue for another, such as lysine for arginine, can also be expected to produce a biologically equivalent product. Nucleotide changes which result in alteration of the N-terminal and

C-terminal portions of the protein molecule would also not be expected to alter the activity of the protein. In some cases, it may in fact be desirable to make mutants of the sequence in order to study the effect of alteration on the biological activity of the protein. Each of the proposed modifications is well within the routine skill in the art, as is determination of retention of biological activity of the encoded products. Moreover, the skilled artisan recognizes that "essentially similar" sequences encompassed by this invention are also defined by their ability to hybridize, under stringent conditions (0.1X SSC, 0.1% SDS, 65°C), with the sequences exemplified herein.

"Gene" refers to a nucleic acid fragment that expresses a specific protein, including regulatory sequences preceding (5' non-coding) and following (3' non-coding) the coding region. "Native" gene refers to the gene as found in nature with its own regulatory sequences. "Chimeric" gene refers to a gene comprising heterogeneous regulatory and coding sequences. "Endogenous" gene refers to the native gene normally found in its natural location in the genome. A "foreign" gene refers to a gene not normally found in the host organism but that is introduced by gene transfer.

"Coding sequence" refers to a DNA sequence that codes for a specific protein and excludes the non-coding sequences.

"Initiation codon" and "termination codon" refer to a unit of three adjacent nucleotides in a coding sequence that specifies initiation and chain termination, respectively, of protein synthesis (mRNA translation). "Open reading frame" refers to the amino acid sequence encoded between translation initiation and termination codons of a coding sequence.

"RNA transcript" refers to the product resulting from RNA polymerase-catalyzed transcription of a DNA sequence. When the RNA transcript is a perfect complementary copy of the DNA sequence, it is referred to as the primary transcript or it may be a RNA sequence derived from posttranscriptional processing of the primary transcript. "Messenger RNA (mRNA) refers to RNA that can be translated into protein by the cell. "cDNA" refers to a double-stranded DNA that is complementary to and derived from mRNA. "Sense" RNA refers to RNA transcript that includes the mRNA. "Antisense RNA" refers to a RNA transcript that is complementary to all or part of a target primary transcript or mRNA and that blocks the expression of a target gene by interfering with the processing, transport and/or translation of its primary transcript or mRNA. The complementarity of an antisense RNA may be with any part of the specific gene transcript, i.e., at the 5' non-coding sequence, 3' non-coding sequence, introns, or the coding sequence. In addition, as used herein, antisense RNA may contain

regions of ribozyme sequences that increase the efficacy of antisense RNA to block gene expression. "Ribozyme" refers to a catalytic RNA and includes sequence-specific endoribonucleases.

As used herein, suitable "regulatory sequences" refer to nucleotide sequences located upstream (5'), within, and/or downstream (3') to a coding sequence, which control the transcription and/or expression of the coding sequences, potentially in conjunction with the protein biosynthetic apparatus of the cell. These regulatory sequences include promoters, translation leader sequences, transcription termination sequences, and polyadenylation sequences.

"Promoter" refers to a DNA sequence in a gene, usually upstream (5') to its coding sequence, which controls the expression of the coding sequence by providing the recognition for RNA polymerase and other factors required for proper transcription. A promoter may also contain DNA sequences that are involved in the binding of protein factors which control the effectiveness of transcription initiation in response to physiological or developmental conditions. It may also contain enhancer elements.

An "enhancer" is a DNA sequence which can stimulate promoter activity. It may be an innate element of the promoter or a heterologous element inserted to enhance the level and/or tissue-specificity of a promoter. "Constitutive promoters" refers to those that direct gene expression in all tissues and at all times. "Organ-specific" or "development-specific" promoters as referred to herein are those that direct gene expression almost exclusively in specific organs, such as leaves or seeds, or at specific development stages in an organ, such as in early or late embryogenesis, respectively.

The term "operably linked" refers to nucleic acid sequences on a single nucleic acid molecule which are associated so that the function of one is affected by the other. For example, a promoter is operably linked with a structure gene (i.e., a gene encoding aspartokinase that is lysine-insensitive as given herein) when it is capable of affecting the expression of that structural gene (i.e., that the structural gene is under the transcriptional control of the promoter).

The term "expression", as used herein, is intended to mean the production of the protein product encoded by a gene. More particularly, "expression" refers to the transcription and stable accumulation of the sense (mRNA) or antisense RNA derived from the nucleic acid fragment(s) of the invention that, in conjunction with the protein apparatus of the cell, results in altered levels of protein product. "Antisense inhibition" refers to the production of antisense RNA transcripts capable of preventing the expression of the target protein. "Overexpression" refers to the production of a gene product in transgenic organisms that exceeds

levels of production in normal or non-transformed organisms. "Cosuppression" refers to the expression of a foreign gene which has substantial homology to an endogenous gene resulting in the suppression of expression of both the foreign and the endogenous gene. "Altered levels" refers to the production of gene product(s) in transgenic organisms in amounts or proportions that differ from that of normal or non-transformed organisms.

The "3' non-coding sequences" refers to the DNA sequence portion of a gene that contains a polyadenylation signal and any other regulatory signal capable of affecting mRNA processing or gene expression. The polyadenylation signal is usually characterized by affecting the addition of polyadenylic acid tracts to the 3' end of the mRNA precursor.

The "translation leader sequence" refers to that DNA sequence portion of a gene between the promoter and coding sequence that is transcribed into RNA and is present in the fully processed mRNA upstream (5') of the translation start codon. The translation leader sequence may affect processing of the primary transcript to mRNA, mRNA stability or translation efficiency.

"Mature" protein refers to a post-translationally processed polypeptide without its targeting signal. "Precursor" protein refers to the primary product of translation of mRNA. A "chloroplast targeting signal" is an amino acid sequence which is translated in conjunction with a protein and directs it to the chloroplast. "Chloroplast transit sequence" refers to a nucleotide sequence that encodes a chloroplast targeting signal.

"Transformation" herein refers to the transfer of a foreign gene into the genome of a host organism and its genetically stable inheritance. Examples of methods of plant transformation include *Agrobacterium*-mediated transformation and particle-accelerated or "gene gun" transformation technology.

"Amino acids" herein refer to the naturally occurring L amino acids (Alanine, Arginine, Aspartic acid, Asparagine, Cystine, Glutamic acid, Glutamine, Glycine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Proline, Phenylalanine, Serine, Threonine, Tryptophan, Tyrosine, and Valine). "Essential amino acids" are those amino acids which cannot be synthesized by animals. A "polypeptide" or "protein" as used herein refers to a molecule composed of monomers (amino acids) linearly linked by amide bonds (also known as peptide bonds).

"Synthetic protein" herein refers to a protein consisting of amino acid sequences that are not known to occur in nature. The amino acid sequence may be derived from a consensus of naturally occurring proteins or may be entirely novel.

"Primary sequence" refers to the connectivity order of amino acids in a polypeptide chain without regard to the conformation of the molecule. Primary sequences are written from the amino terminus to the carboxy terminus of the polypeptide chain by convention.

"Secondary structure" herein refers to physico-chemically favored regular backbone arrangements of a polypeptide chain without regard to variations in side chain identities or conformations. "Alpha helices" as used herein refer to right-handed helices with approximately 3.6 residues per turn of the helix. An "amphipathic helix" refers herein to a polypeptide in a helical conformation where one side of the helix is predominantly hydrophobic and the other side is predominantly hydrophilic.

"Coiled-coil" herein refers to an aggregate of two parallel right-handed alpha helices which are wound around each other to form a left-handed superhelix.

"Salt bridges" as discussed here refer to acid-base pairs of charged amino acid side chains so arranged in space that an attractive electrostatic interaction is maintained between two parts of a polypeptide chain or between one chain and another.

"Host cell" means the cell that is transformed with the introduced genetic material.

Isolation of AK Genes

The *E. coli* lysC gene has been cloned, restriction endonuclease mapped and sequenced previously [Cassan et al. (1986) *J. Biol. Chem. 26*1:1052-1057]. For the present invention the lysC gene was obtained on a bacteriophage lambda clone from an ordered library of 3400 overlapping segments of cloned *E. coli* DNA constructed by Kohara, Akiyama and Isono [Kohara et al. (1987) *Cell 50*:595-508]. The *E. coli* lysC gene encodes the enzyme AKIII, which is sensitive to lysine inhibition. Mutations were obtained in the lysC gene that cause the AKIII enzyme to be resistant to lysine.

To determine the molecular basis for lysine-resistance, the sequence of the wild type lysC gene and three mutant genes were determined. The sequence of the cloned wild type lysC gene, indicated in SEQ ID NO:1:, differed from the published lysC sequence in the coding region at 5 positions.

The sequences of the three mutant lysC genes that encoded lysine-insensitive aspartokinase each differed from the wild type sequence by a single nucleotide, resulting in a single amino acid substitution in the protein. One mutant (M2) had an A substituted for a G at nucleotide 954 of SEQ ID NO:1: resulting in an isoleucine for methionine substitution in the amino acid sequence of AKIII and two mutants (M3 and M4) had identical T for C substitutions at

nucleotide 1055 of SEQ ID NO:1 resulting in an isoleucine for threonine substitution.

Other mutations could be generated, either *in vivo* as described in Example 1 or *in vitro* by site-directed mutagenesis by methods known to those skilled in the art, that result in amino acid substitutions for the methionine or threonine residue present in the wild type AKIII at these positions. Such mutations would be expected to result in a lysine-insensitive enzyme. Furthermore, the method described in Example 1 could be used to easily isolate and characterize as many additional mutant lysC genes encoding lysine insensitive AKIII as desired.

A number of other AK genes have been isolated and sequenced. These include the thrA gene of *E. coli* (Katinka et al. (1980) *Proc. Natl. Acad. Sci. USA 77*:5730-5733], the metL gene of *E. coli* (Zakin et al. (1983) *J. Biol. Chem. 258*:3028-3031], the HOM3 gene of *S. cerevisiae* [Rafalski et al. *(1988) J. Biol. Chem. 263*:2146-2151]. The thrA gene of *E. coli* encodes a bifunctional protein, AKI-HDHI. The AK activity of this enzyme is insensitive to lysine, but sensitive to threonine. The metL gene of *E. coli* also encodes a bifunctional protein, AKII-HDHII, and the AK activity of this enzyme is also insensitive to lysine. The HOM3 gene of yeast encodes an AK which is insensitive to lysine, but sensitive to threonine.

In addition to these genes, several plant genes encoding lysine-insensitive AK are known. In barley lysine plus threonine-resistant mutants bearing mutations in two unlinked genes that result in two different lysine-insensitive AK isoenzymes have been described [Bright et al. (1982) *Nature 299*:278-279, Rognes et al. (1983) *Planta 157*:32-38, Arruda et al. (1984) *Plant Physiol. 76*:442-446]. In corn, a lysine plus threonine-resistant cell line had AK activity that was less sensitive to lysine inhibition than its parent line [Hibberd et al. (1980) *Planta 148*:183-187]. A subsequently isolated lysine plus threonine-resistant corn mutant is altered at a different genetic locus and also produces lysine-insensitive AK [Diedrick et al. (1990) *Theor. Appl. Genet. 79:*209-215, Dotson et al. (1990) *Planta 182*:546-552]. In tobacco there are two AK enzymes in leaves, one lysine-sensitive and one threonine-sensitive. A lysine plus threonine-resistant tobacco mutant that expressed completely lysine-insensitive AK has been described [Frankard et al. (1991) *Theor. Appl. Genet. 82*:273-282]. These plant mutants could serve as sources of genes encoding lysine-insensitive AK and used, based on the teachings herein, to increase the accumulation of lysine and threonine in the seeds of transformed plants.

A partial amino acid sequence of AK from carrot has been reported [Wilson et al. (1991) *Plant Physiol. 97*:1323:1328]. Using this information a set of

degenerate DNA oligonucleotides could be designed, synthesized and used as hybridization probes to permit the isolation of the carrot AK gene. Recently the carrot AK gene has been isolated and its nucleotide sequence has been determined [Matthews et al. (1991) U.S.S.N. 07/746,705]. This gene can be used as a heterologous hybridization probe to isolate the genes encoding lysine-insensitive AK described above.

High level expression of wild type and mutant lysC genes in *E. coli*

To achieve high level expression of the lysC genes in *E. coli,* a bacterial expression vector which employs the bacteriophage T7 RNA polymerase/T7 promoter system [Rosenberg et al. (1987) *Gene 56*:125-135] was used. The expression vector and lysC gene were modified as described in Example 2 to construct a lysC expression vector. For expression of the mutant lysC genes (M2, M3 and M4), the wild type lysC gene was replaced with the mutant genes as described in Example 2.

For high level expression, each of the expression vectors was transformed into *E. coli* strain Bl21(DE3) [Studier et al. (1986) *J. Mol. Biol. 189*:113-130]. Cultures were grown, expression was induced, cells were collected, and extracts were prepared as described in Example 2. Supernatant and pellet fractions of extracts from uninduced and induced cultures were analyzed by SDS polyacrylamide gel electrophoresis and by AK enzyme assays as described in Example 2. The major protein visible by Coomassie blue staining in the supernatant and pellet fractions of induced cultures was AKIII. About 80% of the AKIII protein was in the supernatant and AKIII represented 10-20% of the total *E. coli* protein in the extract.

Approximately 80% of the AKIII enzyme activity was in the supernatant fraction. The specific activity of wild type and mutant crude extracts was 5-7 μmoles product per minute per milligram total protein. Wild type AKIII was sensitive to the presence of L-lysine in the assay. Fifty percent inhibition was found at a concentration of about 0.4 mM and 90 percent inhibition at about 0.1 mM. In contrast, mutants AKIII-M2, M3 and M4 were not inhibited at all by 15 mM L-lysine.

Wild type AKIII protein was purified from the supernatant of an induced culture as described in Example 2. Rabbit antibodies were raised against the purified AKIII protein.

Many other microbial expression vectors have been described in the literature. One skilled in the art could make use of any of these to construct lysC expression vectors. These lysC expression vectors could then be introduced into

appropriate microorganisms via transformation to provide a system for high level expression of AKIII.

Isolation of DHDPS genes

The *E. coli* dapA gene (ecodapA) has been cloned, restriction endonuclease mapped and sequenced previously [Richaud et al. (1986) *J. Bacteriol. 166*:297-300]. For the present invention the dapA gene was obtained on a bacteriophage lambda clone from an ordered library of 3400 overlapping segments of cloned *E. coli.* DNA constructed by Kohara, Akiyama and Isono [Kohara et al. (1987) *Cell 50*:595-508]. The ecodapA gene encodes a DHDPS enzyme that is sensitive to lysine inhibition. However, it is about 20-fold less sensitive to inhibition by lysine than a typical plant DHDPS, e.g., wheat germ DHDPS.

The *Corynebacterium* dapA gene (cordapA) was isolated from genomic DNA from ATCC strain 13032 using polymerase chain reaction (PCR). The nucleotide sequence of the *Corynebacterium* dapA gene has been published [Bonnassie et al. (1990) *Nucleic Acids Res. 18*:6421]. From the sequence it was possible to design oligonucleotide primers for polymerase chain reaction (PCR) that would allow amplification of a DNA fragment containing the gene, and at the same time add unique restriction endonuclease sites at the start codon and just past the stop codon of the gene to facilitate further constructions involving the gene. The details of the isolation of the cordapA gene are presented in Example 3. The cordapA gene encodes a DHDPS enzyme that is insensitive to lysine inhibition.

In addition to introducing a restriction endonuclease site at the translation start codon, the PCR primers also changed the second codon of the cordapA gene from AGC coding for serine to GCT coding for alanine. Several cloned DNA fragments that expressed active, lysine-insensitive DHDPS were isolated, indicating that the second codon amino acid substitution did not affect enzyme activity.

The PCR-generated *Corynebacterium* dapA gene was subcloned into the phagemid vector pGEM-9zf(-) from Promega, and single-stranded DNA was generated and sequenced (SEQ ID NO:6). Aside from the differences in the second codon already mentioned, the sequence matched the published sequence except at two positions, nucleotides 798 and 799. In the published sequence these are TC, while in the gene shown in SEQ ID NO:6 they are CT. This change results in an amino acid substitution of leucine for serine. The reason for this difference is not known. The difference has no apparent effect on DHDPS enzyme activity.

The isolation of other genes encoding DHDPS has been described in the literature. A cDNA encoding DHDPS from wheat [Kaneko et al. (1990) *J. Biol. Chem. 265*:17451-17455], and a cDNA encoding DHDPS from corn [Frisch et al. (1991) *Mol. Gen. Genet. 228*:287-293] are two examples. These genes encode wild type lysine-sensitive DHDPS enzymes. However, Negrutui et al. [(1984) *Theor. Appl. Genet. 68*:11-20], obtained two AEC-resistant tobacco mutants in which DHDPS activity was less sensitive to lysine inhibition than the wild type enzyme. These genes could be isolated using the methods already described for isolating the wheat or corn genes or, alternatively, by using the wheat or corn genes as heterologous hybridization probes.

Still other genes encoding DHDPS could be isolated by one skilled in the art by using either the ecodapA gene, the cordapA gene, or either of the plant DHDPS genes as DNA hybridization probes. Alternatively, other genes encoding DHDPS could be isolated by functional complementation of an *E. coli* dapA mutant, as was done to isolate the cordapA gene [Yeh et al. (1988) *Mol. Gen. Genet. 212*:105-111] and the corn DHDPS gene.

High level expression of ecodapA and cordapA genes in *E. coli*

To achieve high level expression of the ecodapA and cordapA genes in *E. coli*, a bacterial expression vector which employs the bacteriophage T7 RNA polymerase/T7 promoter system [Rosenberg et al. (1987) *Gene 56*:127-135] was used. The vector and dapA genes were modified as described below to construct ecodapA and cordapA expression vectors.

For high level expression each of the expression vectors was transformed into *E. coli* strain BL21(DE3) [Studier et al. (1986) *J. Mol. Biol. 189*:113-130]. Cultures were grown, expression was induced, cells were collected, and extracts were prepared as described in Example 4. Supernatant and pellet fractions of extracts from uninduced and induced cultures were analyzed by SDS polyacrylamide gel electrophoresis and by DHDPS enzyme assays as described in Example 4. The major protein visible by Coomassie blue staining in the supernatant and pellet fractions of both induced cultures had a molecular weight of 32-34 kd, the expected size for DHDPS. Even in the uninduced cultures this protein was the most prominent protein produced.

In the induced culture with the ecodapA gene about 80% of the DHDPS protein was in the supernatant and DHDPS represented 10-20% of the total protein in the extract. In the induced culture with the cordapA gene more than 50% of the DHDPS protein was in the pellet fraction. The pellet fractions in both cases were 90-95% pure DHDPS, with no other single protein present in

significant amounts. Thus, these fractions were pure enough for use in the generation of rabbit antibodies.

The specific activity of *E. coli* DHDPS in the supernatant fraction of induced extracts was about 50 OD_{540} units per milligram protein. *E. coli* DHDPS was sensitive to the presence of L-lysine in the assay. Fifty percent inhibition was found at a concentration of about 0.5 mM. For *Corynebacterium* DHDPS, enzyme activity was measured in the supernatant fraction of uninduced extracts, rather than induced extracts. Enzyme activity was about 4 OD_{530} units per minute per milligram protein. In contrast to *E. coli* DHDPS, *Corynebacterium* DHDPS was not inhibited at all by L-lysine, even at a concentration of 70 mM.

Many other microbial expression vectors have been described in the literature. One skilled in the art could make use of any of these to construct ecodapA or cordapA expression vectors. These expression vectors could then be introduced into appropriate microorganisms via transformation to provide a system for high level expression of DHDPS.

Excretion of amino acids by *E. coli* expressing high levels of DHDPS and/or AKIII

The *E. coli* expression cassettes were inserted into expression vectors and then transformed into *E. coli* strain BL21(DE3) [Studier et al. (1986) *J. Mol. Biol. 189*:113-130] to induce *E. coli* to produce and excrete amino acids. Details of the procedures used and results are presented in Example 5.

Other microbial expression vectors known to those skilled in the art could be used to make and combine expression cassettes for the lysC and dapA genes. These expression vectors could then be introduced into appropriate microorganisms via transformation to provide alternative systems for production and excretion of lysine, threonine and methionine.

Construction of Chimeric Genes for Expression in Plants

A preferred class of heterologous hosts for the expression of the chimeric genes of this invention are eukaryotic hosts, particularly the cells of higher plants. Preferred among the higher plants and the seeds derived from them are soybean, rapeseed (*Brassica napus, B. campestris*), sunflower (*Helianthus annus*), cotton (*Gossypium hirsutum*), corn, tobacco (*Nicotiana tabacum*), alfalfa (*Medicago sativa*), wheat (*Triticum sp*), barley (*Hordeum vulgare*), oats (*Avena sativa, L*), sorghum (*Sorghum* bicolor), rice (*Oryza sativa*), and forage grasses. Expression in plants will use regulatory sequences functional in such plants. The expression of foreign genes in plants is well-established [De Blaere et al. (1987) *Meth. Enzymol. 143*:277-291]. Proper level of expression of the different chimeric genes of this invention in plant cells may be achieved through the use of many

different promoters. Such chimeric genes can be transferred into host plants either together in a single expression vector or sequentially using more than one vector.

The origin of promoter chosen to drive the expression of the coding sequence is not critical as long as it has sufficient transcriptional activity to accomplish the invention by expressing translatable mRNA or antisense RNA in the desired host tissue. Preferred promoters for expression in all plant organs, and especially for expression in leaves include those directing the 19S and 35S transcripts in Cauliflower mosaic virus [Odell et al.(1985) *Nature 313*:810-812; Hull et al. (1987) *Virology 86*:482-493], small subunit of ribulose 1,5-bisphosphate carboxylase [Morelli et al.(1985) *Nature 315*:200; Broglie et al. (1984) *Science 224*:838; Hererra-Estrella et al.(1984) *Nature 310*:115; Coruzzi et al.(1984) *EMBO J. 3*:1671; Faciotti et al.(1985) *Bio/Technology 3*:241], maize zein protein [Matzke et al.(1984) *EMBO J. 3*:1525], and chlorophyll a/b binding protein [Lampa et al.(1986) *Nature 316*:750-752].

Depending upon the application, it may be desirable to select promoters that are specific for expression in one or more organs of the plant. Examples include the light-inducible promoters of the small subunit of ribulose 1,5-bisphosphate carboxylase, if the expression is desired in photosynthetic organs, or promoters active specifically in seeds.

Preferred promoters are those that allow expression specifically in seeds. This may be especially useful, since seeds are the primary source of vegetable amino acids and also since seed-specific expression will avoid any potential deleterious effect in non-seed organs. Examples of seed-specific promoters include, but are not limited to, the promoters of seed storage proteins. The seed storage proteins are strictly regulated, being expressed almost exclusively in seeds in a highly organ-specific and stage-specific manner [Higgins et al.(1984) *Ann. Rev. Plant Physiol. 35*:191-221; Goldberg et al.(1989) *Cell 56*:149-160; Thompson et al. (1989) *BioEssays 10*:108-113]. Moreover, different seed storage proteins may be expressed at different stages of seed development.

There are currently numerous examples for seed-specific expression of seed storage protein genes in transgenic dicotyledonous plants. These include genes from dicotyledonous plants for bean β-phaseolin [Sengupta-Goplalan et al. (1985) *Proc. Natl. Acad. Sci. USA 82*:3320-3324; Hoffman et al. (1988) *Plant Mol. Biol. 11*:717-729], bean lectin [Voelker et al. (1987) *EMBO J. 6*: 3571-3577], soybean lectin [Okamuro et al. (1986) *Proc. Natl. Acad. Sci. USA 83*:8240-8244], soybean kunitz trypsin inhibitor [Perez-Grau et al. (1989) *Plant Cell 1*:095-1109], soybean β-conglycinin [Beachy et al. (1985) *EMBO J. 4*:3047-3053; Barker et al. (1988) *Proc. Natl. Acad. Sci. USA 85*:458-462; Chen et al. (1988) *EMBO J. 7*:297-302;

Chen et al. (1989) *Dev. Genet. 10*:112-122; Naito et al. (1988) *Plant Mol. Biol. 11*:109-123], pea vicilin [Higgins et al. (1988) *Plant Mol. Biol. 11*:683-695], pea convicilin [Newbigin et al. (1990) *Planta 180*:461], pea legumin [Shirsat et al. (1989) *Mol. Gen. Genetics 215*:326]; rapeseed napin [Radke et al. (1988) *Theor. Appl. Genet. 75*:685-694] as well as genes from monocotyledonous plants such as for maize 15 kD zein [Hoffman et al. (1987) *EMBO J. 6*:3213-3221; Schernthaner et al. (1988) *EMBO J. 7*:1249-1253; Williamson et al. (1988) *Plant Physiol. 88*:1002-1007], barley β-hordein [Marris et al. (1988) *Plant Mol. Biol. 10*:359-366] and wheat glutenin [Colot et al. (1987) *EMBO J. 6*:3559-3564]. Moreover, promoters of seed-specific genes, operably linked to heterologous coding sequences in chimeric gene constructs, also maintain their temporal and spatial expression pattern in transgenic plants. Such examples include *Arabidopsis thaliana* 2S seed storage protein gene promoter to express enkephalin peptides in *Arabidopsis* and *B. napus* seeds [Vandekerckhove et al. (1989) *Bio/Technology 7*:929-932], bean lectin and bean β-phaseolin promoters to express luciferase [Riggs et al. (1989) *Plant Sci. 63*:47-57], and wheat glutenin promoters to express chloramphenicol acetyl transferase [Colot et al. (1987) *EMBO J. 6*:3559-3564].

Of particular use in the expression of the nucleic acid fragment of the invention will be the heterologous promoters from several extensively-characterized soybean seed storage protein genes such as those for the Kunitz trypsin inhibitor [Jofuku et al. (1989) *Plant Cell 1*:1079-1093; Perez-Grau et al. (1989) *Plant Cell 1*:1095-1109], glycinin [Nielson et al. (1989) *Plant Cell 1*:313-328], β-conglycinin [Harada et al. (1989) *Plant Cell 1*:415-425]. Promoters of genes for α'- and β-subunits of soybean β-conglycinin storage protein will be particularly useful in expressing mRNAs or antisense RNAs in the cotyledons at mid- to late-stages of soybean seed development [Beachy et al. (1985) *EMBO J. 4*:3047-3053; Barker et al. (1988) *Proc. Natl. Acad. Sci. USA 85*:458-462; Chen et al. (1988) *EMBO J. 7*:297-302; Chen et al. (1989) *Dev. Genet. 10*:112-122; Naito et al. (1988) *Plant Mol. Biol. 11*:109-123] in transgenic plants, since: a) there is very little position effect on their expression in transgenic seeds, and b) the two promoters show different temporal regulation: the promoter for the α'–subunit gene is expressed a few days before that for the β-subunit gene.

Also of particular use in the expression of the nucleic acid fragments of the invention will be the heterologous promoters from several extensively characterized corn seed storage protein genes such as endosperm-specific promoters from the 10 kD zein [Kirihara et al. (1988) *Gene 71*:359-370], the 27 kD zein [Prat et al. (1987) *Gene 52*:51-49; Gallardo et al. (1988) *Plant Sci.*

54:211-281], and the 19 kD zein [Marks et al. (1985) *J. Biol. Chem. 260*:16451-16459]. The relative transcriptional activities of these promoters in corn have been reported [Kodrzyck et al. (1989) *Plant Cell 1*:105-114] providing a basis for choosing a promoter for use in chimeric gene constructs for corn. For expression in corn embryos, the strong embryo-specific promoter from the GLB1 gene [Kriz (1989) *Biochemical Genetics 27*:239-251, Wallace et al. (1991) *Plant Physiol. 95*:973-975] can be used.

It is envisioned that the introduction of enhancers or enhancer-like elements into other promoter constructs will also provide increased levels of primary transcription to accomplish the invention. These would include viral enhancers such as that found in the 35S promoter [Odell et al. (1988) *Plant Mol. Biol. 10*:263-272], enhancers from the opine genes [Fromm et al. (1989) *Plant Cell 1*:977-984], or enhancers from any other source that result in increased transcription when placed into a promoter operably linked to the nucleic acid fragment of the invention.

Of particular importance is the DNA sequence element isolated from the gene for the α'-subunit of β-conglycinin that can confer 40-fold seed-specific enhancement to a constitutive promoter [Chen et al. (1988) *EMBO J. 7*:297-302; Chen et al. (1989) *Dev. Genet. 10*:112-122]. One skilled in the art can readily isolate this element and insert it within the promoter region of any gene in order to obtain seed-specific enhanced expression with the promoter in transgenic plants. Insertion of such an element in any seed-specific gene that is expressed at different times than the β-conglycinin gene will result in expression in transgenic plants for a longer period during seed development.

Any 3' non-coding region capable of providing a polyadenylation signal and other regulatory sequences that may be required for the proper expression can be used to accomplish the invention. This would include the 3' end from any storage protein such as the 3' end of the bean phaseolin gene, the 3' end of the soybean β-conglycinin gene, the 3' end from viral genes such as the 3' end of the 35S or the 19S cauliflower mosaic virus transcripts, the 3' end from the opine synthesis genes, the 3' ends of ribulose 1,5-bisphosphate carboxylase or chlorophyll a/b binding protein, or 3' end sequences from any source such that the sequence employed provides the necessary regulatory information within its nucleic acid sequence to result in the proper expression of the promoter/coding region combination to which it is operably linked. There are numerous examples in the art that teach the usefulness of different 3' non-coding regions [for example, see Ingelbrecht et al. (1989) *Plant Cell 1*:671-680].

DNA sequences coding for intracellular localization sequences may be added to the lysC and dapA coding sequence if required for the proper expression of the proteins to accomplish the invention. Plant amino acid biosynthetic enzymes are known to be localized in the chloroplasts and therefore are synthesized with a chloroplast targeting signal. Bacterial proteins such as DHDPS and AKIII have no such signal. A chloroplast transit sequence could, therefore, be fused to the dapA and lysC coding sequences. Preferred chloroplast transit sequences are those of the small subunit of ribulose 1,5-bisphosphate carboxylase, e.g. from soybean [Berry-Lowe et al. (1982) *J. Mol. Appl. Genet. 1*:483-498] for use in dicotyledonous plants and from corn [Lebrun et al. (1987) *Nucleic Acids Res. 15*:4360] for use in monocotyledonous plants.

Introduction of Chimeric Genes into Plants

Various methods of introducing a DNA sequence (i.e., of transforming) into eukaryotic cells of higher plants are available to those skilled in the art (see EPO publications 0 295 959 A2 and 0 138 341 A1). Such methods include those based on transformation vectors based on the Ti and Ri plasmids of *Agrobacterium spp.* It is particularly preferred to use the binary type of these vectors. Ti-derived vectors transform a wide variety of higher plants, including monocotyledonous and dicotyledonous plants, such as soybean, cotton and rape [Pacciotti et al. (1985) *Bio/Technology 3*:241; Byrne et al. (1987) *Plant Cell, Tissue and Organ Culture 8*:3; Sukhapinda et al. (1987) *Plant Mol. Biol. 8*:209-216; Lorz et al. (1985) *Mol. Gen. Genet. 199*:178; Potrykus (1985) *Mol. Gen. Genet. 199*:183].

For introduction into plants the chimeric genes of the invention can be inserted into binary vectors as described in Examples 7-12 and 14-16. The vectors are part of a binary Ti plasmid vector system [Bevan, (1984) *Nucl. Acids. Res. 12*:8711-8720] of *Agrobacterium tumefaciens*.

Other transformation methods are available to those skilled in the art, such as direct uptake of foreign DNA constructs [see EPO publication 0 295 959 A2], techniques of electroporation [see Fromm et al. (1986) *Nature* (London) *319*:791] or high-velocity ballistic bombardment with metal particles coated with the nucleic acid constructs [see Kline et al. (1987) *Nature* (London) *327*:70, and see U.S. Pat. No. 4,945,050]. Once transformed, the cells can be regenerated by those skilled in the art.

Of particular relevance are the recently described methods to transform foreign genes into commercially important crops, such as rapeseed [see De Block et al. (1989) *Plant Physiol. 91*:694-701], sunflower [Everett et al. (1987) *Bio/Technology 5*:1201], soybean [McCabe et al. (1988) Bio/Technology 6:923; Hinchee et al. (1988) *Bio/Technology 6*:915; Chee et al. (1989) *Plant Physiol.*

91:1212-1218; Christou et al. (1989) Proc. Natl. Acad. Sci USA 86:7500-7504; EPO Publication 0 301 749 A2], and corn [Gordon-Kamm et al. (1990) Plant Cell 2:603-618; Fromm et al. (1990) Biotechnology 8:833-839].

For introduction into plants by high-velocity ballistic bombardment, the chimeric genes of the invention can be inserted into suitable vectors as described in Example 6. Transformed plants can be obtained as described in Examples 17-19.

Expression of lysC and dapA Chimeric Genes in Tobacco Plants

To assay for expression of the chimeric genes in leaves or seeds of the transformed plants, the AKIII or DHDPS proteins can be detected and quantitated enzymatically and/or immunologically by methods known to those skilled in the art. In this way lines producing high levels of expressed protein can be easily identified.

In order to measure the free amino acid composition of the leaves, free amino acids can be extracted by various methods including those as described in Example 7. To measure the free or total amino acid composition of seeds, extracts can be prepared by various methods including those as described in Example 8.

There was no significant effect of expression of AKIII or AKIII-M4 (with a chloroplast targeting signal) on the free lysine or threonine (or any other amino acid) levels in the leaves (see Table 2 in Example 7). Since AKIII-M4 is insensitive to feedback inhibition by any of the end-products of the pathway, this indicates that control must be exerted at other steps in the biosynthetic pathway in leaves.

In contrast, expression of the AKIII or AKIII-M4 (with a chloroplast targeting signal) in the seeds resulted in 2 to 4-fold or 4 to 23-fold increases, respectively, in the level of free threonine in the seeds compared to untransformed plants and 2 to 3-fold increases in the level of free lysine in some cases (Table 3, Example 8). There was a good correlation between transformants expressing higher levels of AKIII or AKIII-M4 protein and those having higher levels of free threonine, but this was not the case for lysine. The relatively small increases of free threonine or lysine achieved with the AKIII protein were not sufficient to yield detectable increases compared to untransformed plants, in the levels of total threonine or lysine in the seeds. The larger increases of free threonine achieved via expression of the AKIII-M4 protein were sufficient to yield detectable increases, compared to seeds from untransformed plants, in the levels of total threonine in the seeds. Sixteen to twenty-five percent increases in total threonine content of the seeds were observed. The lines that showed increased total

threonine were the same ones that showed the highest levels of increase in free threonine and high expression of the AKIII-M4 protein.

The above teachings show that amino acid biosynthesis takes place in seeds and can be modulated by the expression of foreign genes encoding amino acid biosynthetic enzymes. Furthermore, they show that control of an amino acid biosynthetic pathway can differ markedly from one plant organ to another, e.g. seeds and leaves. The importance of this observation is emphasized upon considering the different effects of expressing a foreign DHDPS in leaves and seeds described below. It can be concluded that threonine biosynthesis in seeds is controlled primarily via end-product inhibition of AK. Therefore, threonine accumulation in the seeds of plants can be increased by expression of a gene, introduced via transformation, that encodes AK which is insensitive to lysine inhibition and which is localized in the chloroplast.

The above teachings also demonstrate that transformed plants which express higher levels of the introduced enzyme in seeds accumulate higher levels of free threonine in seeds. Furthermore, the teachings demonstrate that transformed plants which express a lysine-insensitive AK in seeds accumulate higher levels of free threonine in seeds than do transformed plants which express similar levels of a lysine-sensitive AK. To achieve commercially valuable increases in free threonine, a lysine-insensitive AK is preferred.

These teachings indicate that the level of free lysine in seeds controls the accumulation of another aspartate-derived amino acid, threonine, through end-product inhibition of AK. In order to accumulate high levels of free lysine itself, it will be necessary to bypass lysine inhibition of AK via expression of a lysine-insensitive AK.

Expression of active *E. coli* DHDPS enzyme was achieved in both young and mature leaves of the transformed tobacco plants (Table 4, Example 9). High levels of free lysine, 50 to 100-fold higher than normal tobacco plants, accumulated in the young leaves of the plants expressing the enzyme with a chloroplast targeting signal, but not without such a targeting signal. However, a much smaller accumulation of free lysine (2 to 8-fold) was seen in the larger leaves. Experiments that measure lysine in the phloem suggest that lysine is exported from the large leaves. This exported lysine may contribute to the accumulation of lysine in the small growing leaves, which are known to take up, rather than export nutrients. No effect on the free lysine levels in the seeds of these plants was observed even though *E. coli* DHDPS enzyme was expressed in the seeds as well as the leaves.

High level seed-specific expression of *E. coli* DHDPS enzyme, either with or without a chloroplast targeting signal, had no effect on the total, or free, lysine or threonine (or any other amino acid) composition of the seeds in any transformed line (Table 5, Example 10). These results demonstrate that expression in seeds of a DHDPS enzyme that is substantially insensitive to lysine inhibition is not sufficient to lead to increased production or accumulation of free lysine.

These teachings from transformants expressing the *E. coli* DHDPS enzyme indicate that lysine biosynthesis in leaves is controlled primarily via end-product inhibition of DHDPS, while in seeds there must be at least one additional point of control in the pathway. The teachings from transformants expressing the *E. coli* AKIII and AKIII-M4 enzymes indicate that the level of free lysine in seeds controls the accumulation of all aspartate-derived amino acids through end-product inhibition of AK. AK is therefore an additional control point.

To achieve simultaneous, high level expression of both *E. coli* DHDPS and AKIII-M4 in leaves and seeds, plants that express each of the genes could be crossed and hybrids that express both could be selected. Another method would be to construct vectors that contain both genes on the same DNA fragment and introduce the linked genes into plants via transformation. This is preferred because the genes would remain linked throughout subsequent plant breeding efforts. Representative vectors carrying both genes on the same DNA fragment are described in Examples 11, 12, 15, 16, 18, 19, and 25.

Tobacco plants transformed with a vector carrying both *E. coli* DHDPS and AKIII-M4 genes linked to the 35S promoter are described in Example 11. In transformants that express little or no AKIII-M4, the level of expression of *E. coli* DHDPS determines the level of lysine accumulation in leaves (Example 11, Table 6). However, in transformants that express both AKIII-M4 and *E. coli* DHDPS, the level of expression of each protein plays a role in controlling the level of lysine accumulation. Transformed lines that express DHDPS at comparable levels accumulate more lysine when AKIII-M4 is also expressed (Table 6, compare lines 564-18A, 564-56A, 564-36E, 564-55B, and 564-47A). Thus, expression of a lysine-insensitive AK increases lysine accumulation in leaves when expressed in concert with a DHDPS enzyme that is 20-fold less sensitive to lysine than the endogenous plant enzyme.

These leaf results, taken together with the seed results derived from expressing *E. coli* AKIII-M4 and *E. coli* DHDPS separately in seeds, suggest that simultaneous expression of both *E. coli* AKIII-M4 and *E. coli* DHDPS in seeds would lead to increased accumulation of free lysine and would also lead to an

increased accumulation of free threonine. Tobacco plants transformed with a vector carrying both *E. coli* DHDPS and AKIII-M4 genes linked to the phaseolin promoter are described in Example 12. There is an increased accumulation of free lysine and free threonine in these plants. The increased level of free threonine was 4-fold over normal seeds, rather than the 20-fold increase seen in seeds expressing AKIII-M4 alone. The reduction in accumulation of free threonine indicates that pathway intermediates are being diverted down the lysine branch of the biosynthetic pathway. The increased level of free lysine was 2-fold over normal seeds (or seeds expressing *E. coli* DHDPS alone). However, the lysine increase in seeds is not equivalent to the 100-fold increase seen in leaves.

The *E. coli* DHDPS enzyme is less sensitive to lysine inhibition than plant DHDPS, but is still inhibited by lysine. The above teachings on the AK proteins indicate that expression of a completely lysine-insensitive enzyme can lead to a much greater accumulation of the aspartate pathway end-product threonine than expression of an enzyme which, while less sensitive than the plant enzyme, is still inhibited by lysine. Therefore vectors carrying both *Corynebacterium* DHDPS and AKIII-M4 genes linked to the seed-specific promoters were constructed as described in Examples 15 and 19. Tobacco plants transformed with vectors carrying both *Corynebacterium* DHDPS and AKIII-M4 genes linked to seed-specific promoters are described in Example 15. As shown in Table 9, these plants did not show a greater accumulation of free lysine in seeds than previously described plants expressing the *E. coli* DHDPS enzyme in concert with the lysine-insensitive AK. In hindsight this result can be explained by the fact that lysine accumulation in seeds never reached a level high enough to inhibit the *E. coli* DHDPS, so replacement of this enzyme with lysine-insensitive *Corynebacterium* DHDPS had no effect.

In transformed lines expressing high levels of *E. coli* AKIII-M4 and *E. coli* DHDPS or *Corynebacterium* DHDPS, it was possible to detect substantial amounts of α-aminoadipic acid in seeds. This compound is thought to be an intermediate in the catabolism of lysine in cereal seeds, but is normally detected only via radioactive tracer experiments due to its low level of accumulation. The discovery of high levels of this intermediate, comparable to levels of free amino acids, indicates that a large amount of lysine is being produced in the seeds of these transformed lines and is entering the catabolic pathway. The build-up of α-aminoadipic acid was not observed in transformants expressing only *E. coli* DHDPS or only AKIII-M4 in seeds. These results show that it is necessary to express both enzymes simultaneously to produce high levels of free lysine in seeds. To accumulate high levels of free lysine it may also be necessary to

prevent lysine catabolism. Alternatively, it may be desirable to convert the high levels of lysine produced into a form that is insensitive to breakdown, e.g. by incorporating it into a di-, tri- or oligopeptide, or a lysine-rich storage protein.

Expression of lysC and dapA Chimeric Genes
in Rapeseed and Soybean Plants

To analyze for expression of the chimeric lysC and dapA genes in seeds of transformed rapeseed and soybean and to determine the consequences of expression on the amino acid content in the seeds, a seed meal can be prepared as described in Examples 16 or 19 or by any other suitable method. The seed meal can be partially or completely defatted, via hexane extraction for example, if desired. Protein extracts can be prepared from the meal and analyzed for AK and/or DHDPS enzyme activity. Alternatively the presence of the AK and/or DHDPS protein can be tested for immunologically by methods well-known to those skilled in the art. To measure free amino acid composition of the seeds, free amino acids can be extracted from the meal and analyzed by methods known to those skilled in the art (see Examples 8, 16 and 19 for suitable procedures).

All of the rapeseed transformants obtained from a vector carrying the cordapA gene expressed the *Corynebacterium* DHDPS protein, and six of eight transformants obtained from a vector carrying the lysC-M4 gene expressed the AKIII-M4 protein (Example 16, Table 12). Thus it is straightforward to express these proteins in oilseed rape seeds. Transformants expressing DHDPS protein showed a greater than 100-fold increase in free lysine level in their seeds. There was a good correlation between transformants expressing higher levels of DHDPS protein and those having higher levels of free lysine. One transformant that expressed AKIII-M4 in the absence of *Corynebacteria* DHDPS showed a 5-fold increase in the level of free threonine in the seeds. Concomitant expression of both enzymes resulted in accumulation of high levels of free lysine, but not threonine.

A high level of α-aminoadipic acid, indicative of lysine catabolism, was observed in many of the transformed lines, especially lines expressing the highest levels of DHDPS and AKIII protein. Thus, prevention of lysine catabolism by inactivation of lysine ketoglutarate reductase should further increase the accumulation of free lysine in the seeds. Alternatively, incorporation of lysine into a peptide or lysine-rich protein would prevent catabolism and lead to an increase in the accumulation of lysine in the seeds.

To measure the total amino acid composition of mature rapeseed seeds, defatted meal was analyzed as described in Example 16. Relative amino acid levels in the seeds were compared as percentages of lysine to total amino acids.

Seeds with a 5-100% increase in the lysine level, compared to the untransformed control, were observed. The transformant with the highest lysine content expressed high levels of both *E. coli* AKIII-M4 and *Corynebacterium* DHDPS. In this transformant lysine makes up about 13% of the total seed amino acids, considerably higher than any previously known rapeseed seed.

Six of seven soybean transformants expressed the DHDPS protein. In the six transformants that expressed DHDPS, there was excellent correlation between expression of GUS and DHDPS in individual seeds. Therefore, the GUS and DHDPS genes are integrated at the same site in the soybean genome. Four of seven transformants expressed the AKIII protein, and again there was excellent correlation between expression of AKIII, GUS and DHDPS in individual seeds. Thus, in these four transformants the GUS, AKIII and DHDPS genes are integrated at the same site in the soybean genome.

Soybean transformants expressing *Corynebacteria* DHDPS alone and in concert with *E. coli* AKIII-M4 accumulated high levels of free lysine in their seeds. A high level of saccharopine, the first metabolic product of lysine catabolism, was also observed in seeds that contained high levels of lysine. Lesser amounts of α-amino adipic acid were also observed. Thus, prevention of lysine catabolism by inactivation of lysine ketoglutarate reductase should further increase the accumulation of free lysine in the soybean seeds. Alternatively, incorporation of lysine into a peptide or lysine-rich protein would prevent catabolism and lead to an increase in the accumulation of lysine in the soybean seeds.

Analyses of free lysine levels in individual seeds from transformants in which the transgenes segregated as a single locus revealed that the increase in free lysine level was significantly higher in about one-fourth of the seeds. Since one-fourth of the seeds are expected to be homozygous for the transgene, it is likely that the higher lysine seeds are the homozygotes. Furthermore, this indicates that the level of increase in free lysine is dependent upon the transgene copy number. Therefore, lysine levels could be further increased by making hybrids of two different transformants, and obtaining progeny that are homozygous at both transgene loci.

The soybean seeds expressing *Corynebacteria* DHDPS showed substantial increases in accumulation of total seed lysine. Seeds with a 5-35% increase in total lysine content, compared to the untransformed control, were observed. In these seeds lysine makes up 7.5-7.7% of the total seed amino acids.

Soybean seeds expressing *Corynebacteria* DHDPS in concert with *E. coli* AKIII-M4 showed much greater accumulation of total seed lysine than those

expressing *Corynebacteria* DHDPS alone. Seeds with a more than four-fold increase in total lysine content were observed. In these seeds lysine makes up 20-25% of the total seed amino acids, considerably higher than any previously known soybean seed.

Expression of lysC and dapA Chimeric Genes
in Corn Plants

Corn plants regenerated from transformed callus can be analyzed for the presence of the intact lysC and dapA transgenes via Southern blot or PCR. Plants carrying the genes are either selfed or outcrossed to an elite line to generate F1 seeds. Six to eight seeds are pooled and assayed for expression of the *Corynebacterium* DHDPS protein and the *E. coli* AKIII-M4 protein by western blot analysis. The free amino acid composition and total amino acid composition of the seeds are determined as described above.

Expression of the *Corynebacterium* DHDPS protein, and/or the *E. coli* AKIII-M4 protein can be obtained in the embryo of the seed using regulatory sequences active in the embryo, preferably derived from the globulin 1 gene, or in the endosperm using regulatory sequences active in the endosperm, preferably derived from the glutelin 2 gene or the 10 kD zein gene (see Example 26 for details). Free lysine levels in the seeds is increased from about 1.4% of free amino acids in control seeds to 15-27% in seeds of transformants expressing *Corynebacterium* DHDPS alone from the globulin 1 promoter. The increased free lysine was localized to the embryo in seeds expressing *Corynebacterium* DHDPS from the globulin 1 promoter.

The large increases in free lysine result in significant increases in the total seed lysine content. Total lysine levels can be increased at least 130% in seeds expressing *Corynebacterium* DHDPS from the globulin 1 promoter. Greater increases in free lysine levels can be achieved by expressing *E. coli* AKIII-M4 protein from the globulin 1 promoter in concert with *Corynebacterium* DHDPS.

Lysine catabolism is expected to be much greater in the corn endosperm than the embryo. Thus, to achieve significant lysine increases in the endosperm it is preferable to express both *Corynebacterium* DHDPS and the *E. coli* AKIII-M4 in the endosperm and to reduce lysine catabolism by reducing the level of lysine ketoglutarate reductase as described below.

Isolation of a Plant
Lysine Ketoglutarate Reductase Gene

It may be desirable to prevent lysine catabolism in order to accumulate higher levels of free lysine and to prevent accumulation of lysine breakdown products such as saccharopine and α-amino adipic acid. Evidence indicates that

lysine is catabolized in plants via the saccharopine pathway. The first enzymatic evidence for the existence of this pathway was the detection of lysine ketoglutarate reductase (LKR) activity in immature endosperm of developing maize seeds [Arruda et al. (1982) *Plant Physiol. 69*:988-989]. LKR catalyzes the first step in lysine catabolism, the condensation of L-lysine with α-ketoglutarate into saccharopine using NADPH as a cofactor. LKR activity increases sharply from the onset of endosperm development in corn, reaches a peak level at about 20 days after pollination, and then declines [Arruda et al. (1983) *Phytochemistry 22*:2687-2689]. In order to prevent the catabolism of lysine it would be desirable to reduce or eliminate LKR expression or activity. This could be accomplished by cloning the LKR gene, preparing a chimeric gene for cosuppression of LKR or preparing a chimeric gene to express antisense RNA for LKR, and introducing the chimeric gene into plants via transformation. Alternatively, plant mutants could be obtained wherein LKR enzyme activity is absent.

Several methods to clone a plant LKR gene are available to one skilled in the art. The protein can be purified from corn endosperm, as described in Brochetto-Braga et al. [(1992) *Plant Physiol. 98*:1139-1147] and used to raise antibodies. The antibodies can then be used to screen an cDNA expression library for LKR clones. Alternatively the purified protein can be used to determine amino acid sequence at the amino-terminal of the protein or from protease derived internal peptide fragments. Degenerate oligonucleotide probes can be prepared based upon the amino acid sequence and used to screen a plant cDNA or genomic DNA library via hybridization.

Another method makes use of an *E. coli* strain that is unable to grow in a synthetic medium containing 20 μg/mL of L-lysine. Expression of LKR full-length cDNA in this strain will reverse the growth inhibition by reducing the lysine concentration. Construction of a suitable *E. coli* strain and its use to select clones from a plant cDNA library that lead to lysine-resistant growth is described in Example 20.

Yet another method relies upon homology between plant LKR and saccharopine dehydrogenase. Fungal saccharopine dehydrogenase (glutamate-forming) and saccharopine dehydrogenase (lysine-forming) catalyze the final two steps in the fungal lysine biosynthetic pathway. Plant LKR and fungal saccharopine dehydrogenase (lysine-forming) catalyze both forward and reverse reactions, use identical substrates and use similar co-factors. Similarly, plant saccharopine dehydrogenase (glutamate-forming), which catalyzes the second step in the lysine catabolic pathway, works in both forward and reverse reactions, uses identical substrates and uses similar co-factors as fungal saccharopine

dehydrogenase (glutamate-forming). Several genes for fungal saccharopine dehydrogenases have been isolated and sequenced and are readily available to those skilled in the art [Xuan et al. (1990) *Mol. Cell. Biol. 10*:4795-4806, Feller et al. (1994) *Mol. Cell. Biol. 14*:6411-6418]. These genes could be used as heterologous hybridization probes to identify plant LKR and plant saccharopine dehydrogenase (glutamate-forming) nucleic acid fragments, or alternatively to identify homologous protein coding regions in plant cDNAs.

Biochemical and genetic evidence derived from human and bovine studies has demonstrated that mammalian LKR and saccharopine dehydrogenase (glutamate-forming) enzyme activities are present on a single protein with a monomer molecular weight of about 117,000. This contrasts with the fungal enzymes which are carried on separate proteins, saccharopine dehydrogenase (lysine-forming) with a molecular weight of about 44,000, and saccharopine dehydrogenase (glutamate-forming) with a molecular weight of about 51,000. Plant LKR has been reported to have a molecular weight of about 140,000 indicating that it is like the animal catabolic protein wherein both LKR and saccharopine dehydrogenase (glutamate-forming) enzyme activities are present on a single protein.

Two plant saccharopine dehydrogenase (glutamate-forming) nucleic acid fragments (SEQ ID NOS:102 and 103) containing cDNA derived from *Arabidopsis thaliana* are provided. These were identified as cDNAs that encode proteins homologous to fungal saccharopine dehydrogenase (glutamate-forming). These nucleic acid fragments were used to design and synthesize oligonucleotide primers (SEQ ID NO:108 and SEQ ID NO:109). The primers were synthesized and used for PCR amplification of a 2.24 kb DNA fragment from genomic *Arabidopsis* DNA. This DNA fragment was used to isolate a larger genomic DNA fragment, which included the entire coding region, as well as 5' and 3' flanking regions, via hybridization to a genomic DNA library. The sequence of this genomic DNA fragment is provided (SEQ ID NO:110); oligonucleotides were synthesized based on this sequence and used to isolate a full length cDNA via RT-PCR. The sequence of the full length cDNA (SEQ ID NO:111) is provided. These nucleic acid fragments can be used as hybridization probes to identify and isolate genomic DNA fragments or cDNA fragments encoding both LKR and saccharopine dehydrogenase (glutamate-forming) enzyme activities from any plant desired.

The deduced amino acid sequence of *Arabidopsis* LKR/SDH protein is shown in SEQ ID NO:112. The amino acid sequence shows that in plants LKR and SDH enzyme activities are carried on a single bi-functional protein, and that

the protein lacks an N-terminal targeting sequence indicating that the lysine degradative pathway is located in the plant cell cytosol. The amino acid sequence of *Arabidopsis* LKR/SDH protein was compared to that of other LKR and SDH proteins thus revealing regions of conserved amino acid sequence. Degenerate oligonucleotides can be designed based upon this information and used to amplify genomic or cDNA fragments via PCR from other organisms, preferably plants. As an example of this, SEQ ID NO:113 and SEQ ID NO:114 were designed and used to amplify soybean and corn LKR/SDH cDNA fragments. The sequence of a partial soybean LKR/SDH cDNA is shown in SEQ ID NO:115, and the sequence of a partial corn cDNA is shown in SEQ ID NO:116. These DNA fragments can be used to isolate larger genomic DNA fragments, which include the entire coding region, as well as 5' and 3' flanking regions, via hybridization to corn or soybean genomic DNA or cDNA libraries, as was done for *Arabidopsis*. More complete sequence information from the coding regions for soybean and corn LKR/SDH was obtained using the sequences in SEQ ID NOS:115 and 116 as starting materials in protocols such as 5' RACE and hybridization to cDNA libraries. A near full-length cDNA for soybean LKR/SDH is shown in SEQ ID NO:119, and a near full-length cDNA for corn LKR/SDH is shown in SEQ ID NO:120. A truncated version of the LKR/SDH cDNA from corn is set forth in SEQ ID NO:123.

The deduced partial amino acid sequences of soybean LRK/SDH protein is shown in SEQ ID NOS:117 and 121 and the deduced partial amino acid sequences of corn LKR/SDH protein is shown in SEQ ID NO:118, 122 and 124. These amino acid sequences can be compared to other LKR/SDH protein sequences, e.g., the *Arabidopsis* LKR/SDH protein sequence, thus revealing regions of conserved amino acid sequence. With this information oligonucleotide primers can be designed and synthesized to permit isolation of LKR/SDH genomic or cDNA fragments from any plant source.

The availibility of sequence information for plant LKR/SDH proteins from *Arabidopsis*, soybean, and corn allowed comparisons of those sequences to EST sequences obtained from other plants, including ESTs from rice and wheat. SEQ ID NOS:125 and 127 set forth sequences for partial cDNA clones encoding LKR/SDH from rice, and SEQ ID NO:129 set forth the sequence of a partial cDNA encoding a ffragment of LKR/SDH from wheat. The prdicted protein fragments encoded by the sequences presented in SEQ ID NOS:125, 127 and 129 are set forth in SEQ ID NOS:126, 128 and 130, respectively,

The availability of plant LKR/SDH genes makes it possible to block expression of the LKR/SDH gene in transformed plants. To accomplish this a

chimeric gene designed for cosuppression of LKR can be constructed by linking the LKR gene or gene fragment to any of the plant promoter sequences described above. (See U.S. Patent No. 5,231,020 for methodology to block plant gene expression via cosuppression.) Alternatively, a chimeric gene designed to express antisense RNA for all or part of the LKR gene can be constructed by linking the LKR gene or gene fragment in reverse orientation to any of the plant promoter sequences described above. (See U.S. Patent 5,107,065 for methodology to block plant gene expression via antisense RNA.) Either the cosuppression or antisense chimeric gene can be introduced into plants via transformation. Transformants wherein expression of the endogenous LKR gene is reduced or eliminated are then selected.

Preferred promoters for the chimeric genes would be seed-specific promoters. For soybean, rapeseed and other dicotyledonous plants, strong seed-specific promoters from a bean phaseolin gene, a soybean β-conglycinin gene, glycinin gene, Kunitz trypsin inhibitor gene, or rapeseed napin gene would be preferred. For corn and other monocotyledonous plants, a strong endosperm-specific promoter, e.g., the 10 kD or 27 kD zein promoter, or a strong embryo-specific promoter, e.g., the FLB1 promoter, would be preferred.

Transformed plants containing any of the chimeric LKR genes can be obtained by the methods described above. In order to obtain transformed plants that express a chimeric gene for cosuppression of LKR or antisense LKR, as well as a chimeric gene encoding substantially lysine-insensitive DHDPS, the cosuppression or antisense LKR gene could be linked to the chimeric gene encoding substantially lysine-insensitve DHDPS and the two genes could be introduced into plants via transformation. Alternatively, the chimeric gene for cosuppression of LKR or antisense LKR could be introduced into previously transformed plants that express substantially lysine-insensitive DHDPS, or the cosuppression or antisense LKR gene could be introduced into normal plants and the transformants obtained could be crossed with plants that express substantially lysine-insensitive DHDPS.

The availability of plant LKR/SDH genes makes it possible to express the proteins in heterologous systems. To demonstrate this, a DNA fragment which includes the *Arabidopsis* SDH coding region (SEQ ID NO:119) was generated using PCR primers and ligated into a prokaryotic expression vector. High level expression of *Arabidopsis* SDH was achieved in *E. coli* and the SDH protein has been purified from the bacterial extracts, and used to raise rabbit antibodies to the protein. These antibodies can be used to screen for plant mutants in order to find variants which do not produce LKR/SDH protein, or produce reduced amounts of

the protein compared to the parent plant. The plant mutants that express reduced LKR/SDH protein, or no protein at all, could be crossed with plants that express substantially lysine-insensitive DHDPS.

Design of Lysine-Rich Polypeptides

It may be desirable to convert the high levels of lysine produced into a form that is insensitive to breakdown, e.g., by incorporating it into a di-, tri- or oligopeptide, or a lysine-rich storage protein. No natural lysine-rich proteins are known.

One aspect of this invention is the design of polypeptides which can be expressed *in vivo* to serve as lysine-rich seed storage proteins. Polypeptides are linear polymers of amino acids where the α-carboxyl group of one amino acid is covalently bound to the α-amino group of the next amino acid in the chain. Non-covalent interactions among the residues in the chain and with the surrounding solvent determine the final conformation of the molecule. Those skilled in the art must consider electrostatic forces, hydrogen bonds, Van der Waals forces, hydrophobic interactions, and conformational preferences of individual amino acid residues in the design of a stable folded polypeptide chain [see for example: Creighton, (1984) *Proteins, Structures and Molecular Properties*, W. H. Freeman and Company, New York, pp 133-197, or Schulz et al., (1979) *Principles of Protein Structure*, Springer Verlag, New York, pp 27-45]. The number of interactions and their complexity suggest that the design process may be aided by the use of natural protein models where possible.

The synthetic storage proteins (SSPs) embodied in this invention are chosen to be polypeptides with the potential to be enriched in lysine relative to average levels of proteins in plant seeds. Lysine is a charged amino acid at physiological pH and is therefore found most often on the surface of protein molecules [Chothia, (1976) *Journal of Molecular Biology 105*:1-14]. To maximize lysine content, Applicants chose a molecular shape with a high surface-to-volume ratio for the synthetic storage proteins embodied in this invention. The alternatives were either to stretch the common globular shape of most proteins to form a rod-like extended structure or to flatten the globular shape to a disk-like structure. Applicants chose the former configuration as there are several natural models for long rod-like proteins in the class of fibrous proteins [Creighton, (1984) *Proteins, Structures and Molecular Properties*, W.H. Freeman and Company, New York, p 191].

Coiled-coils constitute a well-studied subset of the class of fibrous proteins [see Cohen et al., (1986) *Trends Biochem. Sci. 11*:245-248]. Natural examples are found in α-keratins, paramyosin, light meromyosin and tropomyosin. These protein molecules consist of two parallel alpha helices twisted about each other in

a left-handed supercoil. The repeat distance of this supercoil is 140 Å (compared to a repeat distance of 5.4 Å for one turn of the individual helices). The supercoil causes a slight skew (10°) between the axes of the two individual alpha helices.

In a coiled coil there are 3.5 residues per turn of the individual helices resulting in an exact 7 residue periodicity with respect to the superhelix axis (see Figure 1). Every seventh amino acid in the polypeptide chain therefore occupies an equivalent position with respect to the helix axis. Applicants refer to the seven positions in this heptad unit of the invention as (d e f g a b c) as shown in Figures 1 and 2a. This conforms to the conventions used in the coiled-coil literature.

The a and d amino acids of the heptad follow a 4,3 repeat pattern in the primary sequence and fall on one side of an individual alpha helix (See Figure 1). If the amino acids on one side of an alpha helix are all non-polar, that face of the helix is hydrophobic and will associate with other hydrophobic surfaces as, for example, the non-polar face of another similar helix. A coiled-coil structure results when two helices dimerize such that their hydrophobic faces are aligned with each other (See Figure 2a).

The amino acids on the external faces of the component alpha helices (b, c, e, f, g) are usually polar in natural coiled-coils in accordance with the expected pattern of exposed and buried residue types in globular proteins [Schulz, et al., (1979) *Principles of Protein Structure*. Springer Verlag, New York, p 12; Talbot, et al , (1982) *Acc. Chem. Res. 15*:224-230; Hodges et al., (1981) *Journal of Biological Chemistry 256*:1214-1224]. Charged amino acids are sometimes found forming salt bridges between positions e and g' or positions g and e' on the opposing chain (see Figure 2a).

Thus, two amphipathic helices like the one shown in Figure 1 are held together by a combination of hydrophobic interactions between the a, a', d, and d' residues and by salt bridges between e and g' and/or g and e' residues. The packing of the hydrophobic residues in the supercoil maintains the chains "in register". For short polypeptides comprising only a few turns of the component alpha helical chains, the 10° skew between the helix axes can be ignored and the two chains treated as parallel (as shown in Figure 2a).

A number of synthetic coiled-coils have been reported in the literature (Lau et al., (1984) *Journal of Biological Chemistry 259*:13253-13261; Hodges et al., (1988) *Peptide Research 1*:19-30; DeGrado et al., (1989) *Science 243*:622-628; O'Neil et al., (1990) *Science 250*:646-651]. Although these polypeptides vary in size, Lau et al. found that 29 amino acids were sufficient for dimerization to form the coiled-coil structure [Lau et al., (1984) *Journal of Biological Chemistry*

259:13253-13261]. Applicants constructed the polypeptides in this invention as 28-residue and larger chains for reasons of conformational stability.

The polypeptides of this invention are designed to dimerize with a coiled-coil motif in aqueous environments. Applicants have used a combination of hydrophobic interactions and electrostatic interactions to stabilize the coiled-coil conformation. Most nonpolar residues are restricted to the a and d positions which creates a hydrophobic stripe parallel to the axis of the helix. This is the dimerization face. Applicants avoided large, bulky amino acids along this face to minimize steric interference with dimerization and to facilitate formation of the stable coiled-coil structure.

Despite recent reports in the literature suggesting that methionine at positions a and d is destabilizing to coiled-coils in the leucine zipper subgroup [Landschulz et al., (1989) *Science 243*:1681-1688 and Hu et al., (1990) *Science 250*:1400-1403], Applicants chose to substitute methionine residues for leucine on the hydrophobic face of the SSP polypeptides. Methionine and leucine are similar in molecular shape (Figure 3). Applicants demonstrated that any destabilization of the coiled-coil that may be caused by methionine in the hydrophobic core appears to be compensated in sequences where the formation of salt bridges (e-g' and g-e') occurs at all possible positions in the helix (i.e., twice per heptad).

To the extent that it is compatible with the goal of creating a polypeptide enriched in lysine, Applicants minimized the unbalanced charges in the polypeptide. This may help to prevent undesirable interactions between the synthetic storage proteins and other plant proteins when the polypeptides are expressed *in vivo*.

The polypeptides of this invention are designed to spontaneously fold into a defined, conformationally stable structure, the alpha helical coiled-coil, with minimal restrictions on the primary sequence. This allows synthetic storage proteins to be custom-tailored for specific end-user requirements. Any amino acid can be incorporated at a frequency of up to one in every seven residues using the b, c, and f positions in the heptad repeat unit. Applicants note that up to 43% of an essential amino acid from the group isoleucine, leucine, lysine, methionine, threonine, and valine can be incorporated and that up to 14% of the essential amino acids from the group phenylalanine, tryptophan, and tyrosine can be incorporated into the synthetic storage proteins of this invention.

In the SSPs only Met, Leu, Ile, Val or Thr are located in the hydrophobic core. Furthermore, the e, g, e', and g' positions in the SSPs are restricted such that an attractive electrostatic interaction always occurs at these positions between the

two polypeptide chains in an SSP dimer. This makes the SSP polypeptides more stable as dimers.

Thus, the novel synthetic storage proteins described in this invention represent a particular subset of possible coiled-coil polypeptides. Not all polypeptides which adopt an amphipathic alpha helical conformation in aqueous solution are suitable for the applications described here.

The following rules derived from Applicants' work define the SSP polypeptides that Applicants use in their invention:

The synthetic polypeptide comprises n heptad units (d e f g a b c), each heptad being either the same or different, wherein:

n is at least 4;

a and d are independently selected from the group consisting of Met, Leu, Val, Ile and Thr;

e and g are independently selected from the group consisting of the acid/base pairs Glu/Lys, Lys/Glu, Arg/Glu, Arg/Asp, Lys/Asp, Glu/Arg, Asp/Arg and Asp/Lys; and

b, c and f are independently any amino acids except Gly or Pro and at least two amino acids of b, c and f in each heptad are selected from the group consisting of Glu, Lys, Asp, Arg, His, Thr, Ser, Asn, Gln, Cys and Ala.

Chimeric Genes Encoding Lysine-Rich Polypeptides

DNA sequences which encode the polypeptides described above can be designed based upon the genetic code. Where multiple codons exist for particular amino acids, codons should be chosen from those preferable for translation in plants. Oligonucleotides corresponding to these DNA sequences can be synthesized using an ABI DNA synthesizer, annealed with oligonucleotides corresponding to the complementary strand and inserted into a plasmid vector by methods known to those skilled in the art. The encoded polypeptide sequences can be lengthened by inserting additional annealed oligonucleotides at restriction endonuclease sites engineered into the synthetic gene. Some representative strategies for constructing genes encoding lysine-rich polypeptides of the invention, as well as DNA and amino acid sequences of preferred embodiments are provided in Example 21.

A chimeric gene designed to express RNA for a synthetic storage protein gene encoding a lysine-rich polypeptide can be constructed by linking the gene to any of the plant promoter sequences described above. Preferred promoters would be seed-specific promoters. For soybean, rapeseed and other dicotyledonous plants strong seed-specific promoters from a bean phaseolin gene, a soybean

β-conglycinin gene, glycinin gene, Kunitz trypsin inhibitor gene, or rapeseed napin gene would be preferred. For corn or other monocotyledonous plants, a strong endosperm-specific promoter, e.g., the 10 kD or 27 kD zein promoter, or a strong embyro-specific promoter, e.g., the corn globulin 1 promoter, would be preferred.

In order to obtain plants that express a chimeric gene for a synthetic storage protein gene encoding a lysine-rich polypeptide, plants can be transformed by any of the methods described above. In order to obtain plants that express both a chimeric SSP gene and chimeric genes encoding substantially lysine-insensitive DHDPS and AK, the SSP gene could be linked to the chimeric genes encoding substantially lysine-insensitive DHDPS and AK and the three genes could be introduced into plants via transformation. Alternatively, the chimeric SSP gene could be introduced into previously transformed plants that express substantially lysine-insensitive DHDPS and AK, or the SSP gene could be introduced into normal plants and the transformants obtained could be crossed with plants that express substantially lysine-insensitive DHDPS and AK.

Results from genetic crosses of transformed plants containing lysine biosynthesis genes with transformed plants containing lysine-rich protein genes (see Example 23) demonstrate that the total lysine levels in seeds can be increased by the coordinate expression of these genes. This result was especially striking because the gene copy number of all of the transgenes was reduced in the hybrid. It is expected that the lysine level would be further increased if the biosynthesis genes and the lysine-rich protein genes were all homozygous.

Use of the cts/lysC-M4 Chimeric Gene as a Selectable Marker for Plant Transformation

Growth of cell cultures and seedlings of many plants is inhibited by high concentrations of lysine plus threonine. Growth is restored by addition of methionine (or homoserine which is converted to methionine *in vivo*). Lysine plus threonine inhibition is thought to result from feedback inhibition of endogenous AK, which reduces flux through the pathway leading to starvation for methionine. In tobacco there are two AK enzymes in leaves, one lysine-sensitive and one threonine sensitive.[Negrutui et al. (1984) *Theor. Appl. Genet. 68*:11-20]. High concentrations of lysine plus threonine inhibit growth of shoots from tobacco leaf disks and inhibition is reversed by addition of low concentrations of methionine. Thus, growth inhibition is presumably due to inhibition of the two AK isozymes.

Expression of active lysine and threonine insensitive AKIII-M4 also reverses lysine plus threonine growth inhibition (Table 2, Example 7). There is a good correlation between the level of AKIII-M4 protein expressed and the

resistance to lysine plus threonine. Expression of lysine-sensitive wild type AKIII does not have a similar effect. Since expression of the AKIII-M4 protein permits growth under normally inhibitory conditions, a chimeric gene that causes expression of AKIII-M4 in plants can be used as a selectable genetic marker for transformation as illustrated in Examples 13 and 17.

EXAMPLES

The present invention is further defined in the following Examples, in which all parts and percentages are by weight and degrees are Celsius, unless otherwise stated. It should be understood that these Examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

EXAMPLE 1

Isolation of the *E. coli* lysC Gene and mutations in lysC resulting in lysine-insensitive AKIII

The *E. coli* lysC gene has been cloned, restriction endonuclease mapped and sequenced previously [Cassan et al. (1986) *J. Biol. Chem. 26*1:1052-1057]. For the present invention the lysC gene was obtained on a bacteriophage lambda clone from an ordered library of 3400 overlapping segments of cloned *E. coli* DNA constructed by Kohara, Akiyama and Isono [Kohara et al. (1987) *Cell 50*:595-508]. This library provides a physical map of the whole *E. coli* chromosome and ties the physical map to the genetic map. From the knowledge of the map position of lysC at 90 min on the *E. coli* genetic map [Theze et al. (1974) *J. Bacteriol. 117*:133-143], the restriction endonuclease map of the cloned gene [Cassan et al. (1986) *J. Biol. Chem. 261*:1052-1057], and the restriction endonuclease map of the cloned DNA fragments in the *E. coli* library [Kohara et al. (1987) *Cell 50*:595-508], it was possible to choose lambda phages 4E5 and 7A4 [Kohara et al. (1987) *Cell 50*:595-508] as likely candidates for carrying the lysC gene. The phages were grown in liquid culture from single plaques as described [see *Current Protocols in Molecular Biology* (1987) Ausubel et al. Eds. John Wiley & Sons New York] using LE392 as host [see Sambrook et al. (1989) *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory Press]. Phage DNA was prepared by phenol extraction as described [see Current Protocols in *Molecular Biology* (1987) Ausubel et al. eds. John Wiley & Sons New York].

From the sequence of the gene several restriction endonuclease fragments diagnostic for the lysC gene were predicted, including an 1860 bp EcoR I-Nhe I fragment, a 2140 bp EcoR I-Xmn I fragment and a 1600 bp EcoR I-BamH I fragment. Each of these fragments was detected in both of the phage DNAs confirming that these carried the lysC gene. The EcoR I-Nhe I fragment was isolated and subcloned in plasmid pBR322 digested with the same enzymes, yielding an ampicillin-resistant, tetracycline-sensitive *E. coli* transformant. The plasmid was designated pBT436.

To establish that the cloned lysC gene was functional, pBT436 was transformed into *E. coli* strain Gif106M1 (E. coli Genetic Stock Center strain CGSC-5074) which has mutations in each of the three *E. coli* AK genes [Theze et al. (1974) *J. Bacteriol. 117*:133-143]. This strain lacks all AK activity and therefore requires diaminopimelate (a precursor to lysine which is also essential for cell wall biosynthesis), threonine and methionine. In the transformed strain all these nutritional requirements were relieved demonstrating that the cloned lysC gene encoded functional AKIII.

Addition of lysine (or diaminopimelate which is readily converted to lysine in vivo) at a concentration of approximately 0.2 mM to the growth medium inhibits the growth of Gif106M1 transformed with pBT436. M9 media [see Sambrook et al. (1989) *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory Press] supplemented with the arginine and isoleucine, required for Gif106M1 growth, and ampicillin, to maintain selection for the pBT436 plasmid, was used. This inhibition is reversed by addition of threonine plus methionine to the growth media. These results indicated that AKIII could be inhibited by exogenously added lysine leading to starvation for the other amino acids derived from aspartate. This property of pBT436-transformed Gif106M1 was used to select for mutations in lysC that encoded lysine-insensitive AKIII.

Single colonies of Gif106M1 transformed with pBT436 were picked and resuspended in 200 μL of a mixture of 100 μL 1% lysine plus 100 μL of M9 media. The entire cell suspension containing 10^7-10^8 cells was spread on a petri dish containing M9 media supplemented with the arginine, isoleucine, and ampicillin. Sixteen petri dishes were thus prepared. From 1 to 20 colonies appeared on 11 of the 16 petri dishes. One or two (if available) colonies were picked and retested for lysine resistance and from this nine lysine-resistant clones were obtained. Plasmid DNA was prepared from eight of these and re-transformed into Gif106M1 to determine whether the lysine resistance determinant was plasmid-borne. Six of the eight plasmid DNAs yielded lysine-resistant colonies. Three of these six carried lysC genes encoding AKIII that was

uninhibited by 15 mM lysine, whereas wild type AKIII is 50% inhibited by 0.3-0.4 mM lysine and >90% inhibited by 1 mM lysine (see Example 2 for details).

To determine the molecular basis for lysine-resistance the sequences of the wild type lysC gene and three mutant genes were determined. A method for "Using mini-prep plasmid DNA for sequencing double stranded templates with Sequenase™" [Kraft et al. (1988) *BioTechniques 6*:544-545] was used. Oligonucleotide primers, based on the published lysC sequence and spaced approximately every 200 bp, were synthesized to facilitate the sequencing. The sequence of the wild type lysC gene cloned in pBT436 (SEQ ID NO:1) differed from the published lysC sequence in the coding region at 5 positions. Four of these nucleotide differences were at the third position in a codon and would not result in a change in the amino acid sequence of the AKIII protein. One of the differences would result in a cysteine to glycine substitution at amino acid 58 of AKIII. These differences are probably due to the different strains from which the lysC genes were cloned.

The sequences of the three mutant lysC genes that encoded lysine-insensitive AK each differed from the wild type sequence by a single nucleotide, resulting in a single amino acid substitution in the protein. Mutant M2 had an A substituted for a G at nucleotide 954 of SEQ ID NO:1 resulting in an isoleucine for methionine substitution at amino acid 318 and mutants M3 and M4 had identical T for C substitutions at nucleotide 1055 of SEQ ID NO:1 resulting in an isoleucine for threonine substitution at amino acid 352. Thus, either of these single amino acid substitutions is sufficient to render the AKIII enzyme insensitive to lysine inhibition.

EXAMPLE 2

High level expression of wild type and mutant lysC genes in *E. coli*

An Nco I (CCATGG) site was inserted at the translation initiation codon of the lysC gene using the following oligonucleotides:

SEQ ID NO:2:
GATCCATGGC TGAAATTGTT GTCTCCAAAT TTGGCG

SEQ ID NO:3:
GTACCGCCAA ATTTGGAGAC AACAATTTCA GCCATG

When annealed these oligonucleotides have BamH I and Asp718 "sticky" ends. The plasmid pBT436 was digested with BamH I, which cuts upstream of the lysC

coding sequence and Asp718 which cuts 31 nucleotides downstream of the initiation codon. The annealled oligonucleotides were ligated to the plasmid vector and *E. coli* transformants were obtained. Plasmid DNA was prepared and screened for insertion of the oligonucleotides based on the presence of an Nco I site. A plasmid containing the site was sequenced to assure that the insertion was correct, and was designated pBT457. In addition to creating an Nco I site at the initiation codon of lysC, this oligonucleotide insertion changed the second codon from TCT, coding for serine, to GCT, coding for alanine. This amino acid substitution has no apparent effect on the AKIII enzyme activity.

To achieve high level expression of the lysC genes in *E. coli,* the bacterial expression vector pBT430 was used. This vector is a derivative of pET-3a [Rosenberg et al. (1987) *Gene 56*:125-135] which employs the bacteriophage T7 RNA polymerase/T7 promoter system. Plasmid pBT430 was constructed by first destroying the EcoR I and Hind III sites in pET-3a at their original positions. An oligonucleotide adaptor containing EcoR I and Hind III sites was inserted at the BamH I site of pET-3a. This created pET-3aM with additional unique cloning sites for insertion of genes into the expression vector. Then, the Nde I site at the position of translation initiation was converted to an Nco I site using oligonucleotide-directed mutagenesis. The DNA sequence of pET-3aM in this region, 5'-CATATGG, was converted to 5'-CCCATGG in pBT430.

The lysC gene was cut out of plasmid pBT457 as a 1560 bp Nco I-EcoR I fragment and inserted into the expression vector pBT430 digested with the same enzymes, yielding plasmid pBT461. For expression of the mutant lysC genes (M2, M3 and M4) pBT461 was digested with Kpn I-EcoR I, which removes the wild type lysC gene from about 30 nucleotides downstream from the translation start codon, and inserting the homologous Kpn I-EcoR I fragments from the mutant genes yielding plasmids pBT490, pBT491 and pBT492, respectively.

For high level expression each of the plasmids was transformed into *E. coli* strain BL21(DE3) [Studier et al. (1986) *J. Mol. Biol. 189*:113-130]. Cultures were grown in LB medium containing ampicillin (100 mg/L) at 25°C. At an optical density at 600 nm of approximately 1, IPTG (isopropylthio-β-galactoside, the inducer) was added to a final concentration of 0.4 mM and incubation was continued for 3 h at 25°. The cells were collected by centrifugation and resuspended in 1/20th (or 1/100th) the original culture volume in 50 mM NaCl; 50 mM Tris-Cl, pH 7.5; 1 mM EDTA, and frozen at -20°. Frozen aliquots of 1 mL were thawed at 37° and sonicated, in an ice-water bath, to lyse the cells. The lysate was centrifuged at 4° for 5 min at 15,000 rpm. The supernatant was removed and the pellet was resuspended in 1 mL of the above buffer.

The supernatant and pellet fractions of uninduced and IPTG-induced cultures of BL21(DE3)/pBT461 were analyzed by SDS polyacrylamide gel electrophoresis. The major protein visible by Coomassie blue staining in the supernatant of the induced culture had a molecular weight of about 48 kd, the expected size for AKIII. About 80% of the AKIII protein was in the supernatant and AKIII represented 10-20% of the total *E. coli* protein in the extract.

AK activity was assayed as shown below:

Assay mix (for 12 assay tubes):

4.5 mL H_20

1.0 mL 8M KOH

1.0 mL 8M NH_2OH-HCl

1.0 mL 1M Tris-HCl pH 8.0

0.5 mL 0.2M ATP (121 mg/mL in 0.2M NaOH)

50 μL 1M $MgSO_4$

Each 1.5 mL eppendorf assay tube contained:

0.64 mL assay mix

0.04 mL 0.2 M L-aspartic acid or 0.04 mL H_2O

0.0005-0.12 mL extract

H_20 to total volume 0.8 mL

Assay tubes were incubated at 30° for desired time (10-60 min). Then 0.4 mL $FeCl_3$ reagent (10% w/v $FeCl_3$, 3.3% trichloroacetic acid, 0.7 M HCl) was added and the material centrifuged for 2 min in an eppendorf centrifuge. The supernatant was decanted. The OD was read at 540 nm and compared to the aspartyl-hydroxamate standard.

Approximately 80% of the AKIII activity was in the supernatant fraction. The specific activity of wild type and mutant crude extracts was 5-7 μM product per min per milligram total protein. Wild type AKIII was sensitive to the presence of L-lysine in the assay. Fifty percent inhibition was found at a concentration of about 0.4 mM and 90% inhibition at about 1.0 mM. In contrast, mutants AKIII-M2, M3 and M4 (see Example 1) were not inhibited at all by 15 mM L-lysine.

Wild type AKIII protein was purified from the supernatant of the IPTG-induced culture as follows. To 1 mL of extract, 0.25 mL of 10% streptomycin sulfate was added and kept at 4° overnight. The mixture was centrifuged at 4° for 15 min at 15,000 rpm. The supernatant was collected and desalted using a Sephadex G-25 M column (Column PD-10, Pharmacia). It was then run on a

Mono-Q HPLC column and eluted with a 0-1M NaCl gradient. The two 1 mL fractions containing most of the AKIII activity were pooled, concentrated, desalted and run on an HPLC sizing column (TSK G3000SW). Fractions were eluted in 20 mM KPO_4 buffer, pH7.2, 2 mM $MgSO_4$, 10 mM β-mercaptoethanol, 0.15 M KCl, 0.5 mM L-lysine and were found to be >95% pure by SDS polyacrylamide gel electrophoresis. Purified AKIII protein was sent to Hazelton Research Facility (310 Swampridge Road, Denver, PA 17517) to have rabbit antibodies raised against the protein.

EXAMPLE 3

Isolation of the *E. coli* and *Corynebacterium glutamicum* dapA genes

The *E. coli* dapA gene (ecodapA) has been cloned, restriction endonuclease mapped and sequenced previously [Richaud et al. (1986) *J. Bacteriol. 166*:297-300]. For the present invention the dapA gene was obtained on a bacteriophage lambda clone from an ordered library of 3400 overlapping segments of cloned *E. coli* DNA constructed by Kohara, Akiyama and Isono [Kohara et al. (1987) *Cell 50*:595-508, see Example 1]. From the knowledge of the map position of dapA at 53 min on the *E. coli* genetic map [Bachman (1983) *Microbiol. Rev. 47*:180-230], the restriction endonuclease map of the cloned gene [Richaud et al. (1986) *J. Bacteriol. 166*:297-300], and the restriction endonuclease map of the cloned DNA fragments in the *E. coli* library [Kohara et al. (1987) *Cell 50*:595-508], it was possible to choose lambda phages 4C11 and 5A8 [Kohara et al. (1987) *Cell 50*:595-508] as likely candidates for carrying the dapA gene. The phages were grown in liquid culture from single plaques as described [see Current Protocols in Molecular Biology (1987) Ausubel et al. eds., John Wiley & Sons New York] using LE392 as host [see Sambrook et al. (1989) *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory Press]. Phage DNA was prepared by phenol extraction as described [see *Current Protocols in Molecular Biology* (1987) Ausubel et al. eds., John Wiley & Sons New York]. Both phages contained an approximately 2.8 kb Pst I DNA fragment expected for the dapA gene [Richaud et al. (1986) *J. Bacteriol. 166*:297-300]. The fragment was isolated from the digest of phage 5A8 and inserted into Pst I digested vector pBR322 yielding plasmid pBT427.

The *Corynebacterium* dapA gene (cordapA) was isolated from genomic DNA from ATCC strain 13032 using polymerase chain reaction (PCR). The nucleotide sequence of the *Corynebacterium* dapA gene has been published [Bonnassie et al. (1990) *Nucleic Acids Res. 18*:6421]. From the sequence it was possible to design oligonucleotide primers for PCR that would allow amplification of a DNA fragment containing the gene, and at the same time add unique

restriction endonuclease sites at the start codon (Nco I) and just past the stop codon (EcoR I) of the gene. The oligonucleotide primers used were:

SEQ ID NO:4:
CCCGGGCCAT GGCTACAGGT TTAACAGCTA AGACCGGAGT AGAGCACT

SEQ ID NO:5:
GATATCGAAT TCTCATTATA GAACTCCAGC TTTTTTC

PCR was performed using a Perkin-Elmer Cetus kit according to the instructions of the vendor on a thermocycler manufactured by the same company. The reaction product, when run on an agarose gel and stained with ethidium bromide, showed a strong DNA band of the size expected for the *Corynebacterium* dapA gene, about 900 bp. The PCR-generated fragment was digested with restriction endonucleases Nco I and EcoR I and inserted into expression vector pBT430 (see Example 2) digested with the same enzymes. In addition to introducing an Nco I site at the translation start codon, the PCR primers also resulted in a change of the second codon from AGC coding for serine to GCT coding for alanine. Several clones that expressed active, lysine-insensitive DHDPS (see Example 4) were isolated, indicating that the second codon amino acid substitution did not affect activity; one clone was designated FS766.

The Nco I to EcoR I fragment carrying the PCR-generated *Corynebacterium* dapA gene was subcloned into the phagemid vector pGEM-9Zf(-) from Promega, single-stranded DNA was prepared and sequenced. This sequence is shown in SEQ ID NO:6.

Aside from the differences in the second codon already mentioned, the sequence matched the published sequence except at two positions, nucleotides 798 and 799. In the published sequence these are TC, while in the gene shown in SEQ ID NO:6 they are CT. This change results in an amino acid substitution of leucine for serine. The reason for this difference is not known. It may be due to an error in the published sequence, the difference in strains used to isolate the gene, or a PCR-generated error. The latter seems unlikely since the same change was observed in at least 3 independently isolated PCR-generated dapA genes. The difference has no apparent effect on DHDPS enzyme activity (see Example 4).

EXAMPLE 4

High level expression of the *E. coli* and *Corynebacterium* glutamicum dapA genes in *E. coli*

An Nco I (CCATGG) site was inserted at the translation initiation codon of the *E. coli* dapA gene using oligonucleotide-directed mutagenesis. The 2.8 kb Pst I DNA fragment carrying the dapA gene in plasmid pBT427 (see Example 3) was inserted into the Pst I site of phagemid vector pTZ18R (Pharmacia) yielding pBT431. The orientation of the dapA gene was such that the coding strand would be present on the single-stranded phagemid DNA. Oligonucleotide-directed mutagenesis was carried out using a Muta-Gene kit from Bio-Rad according to the manufacturer's protocol with the mutagenic primer shown below:

SEQ ID NO:7:
CTTCCCGTGA CCATGGGCCA TC

Putative mutants were screened for the presence of an Nco I site and a plasmid, designated pBT437, was shown to have the proper sequence in the vicinity of the mutation by DNA sequencing. The addition of an Nco I site at the translation start codon also resulted in a change of the second codon from TTC coding for phenylalanine to GTC coding for valine.

To achieve high level expression of the dapA genes in *E. coli* the bacterial expression vector pBT430 (see Example 2) was used. The *E. coli* dapA gene was cut out of plasmid pBT437 as an 1150 bp Nco I-Hind III fragment and inserted into the expression vector pBT430 digested with the same enzymes, yielding plasmid pBT442. For expression of the *Corynebacterium* dapA gene, the 910 bp Nco I to EcoR I fragment of SEQ ID NO:6 inserted in pBT430 (pFS766, see Example 3) was used.

For high level expression each of the plasmids was transformed into *E. coli* strain BL21(DE3) [Studier et al. (1986) *J. Mol. Biol. 189*:113-130]. Cultures were grown in LB medium containing ampicillin (100 mg/L) at 25°. At an optical density at 600 nm of approximately 1, IPTG (isopropylthio-β-galactoside, the inducer) was added to a final concentration of 0.4 mM and incubation was continued for 3 h at 25°. The cells were collected by centrifugation and resuspended in 1/20th (or 1/100th) the original culture volume in 50 mM NaCl; 50 mM Tris-Cl, pH 7.5; 1 mM EDTA, and frozen at -20°. Frozen aliquots of 1 mL were thawed at 37° and sonicated, in an ice-water bath, to lyse the cells. The lysate was centrifuged at 4° for 5 min at 15,000 rpm. The supernatant was removed and the pellet was resuspended in 1 mL of the above buffer.

The supernatant and pellet fractions of uninduced and IPTG-induced cultures of BL21(DE3)/pBT442 or BL21(DE3)/pFS766 were analyzed by SDS polyacrylamide gel electrophoresis. The major protein visible by Coomassie blue staining in the supernatant and pellet fractions of both induced cultures had a molecular weight of 32-34 kd, the expected size for DHDPS. Even in the uninduced cultures this protein was the most prominent protein produced.

In the BL21(DE3)/pBT442 IPTG-induced culture about 80% of the DHDPS protein was in the supernatant and DHDPS represented 10-20% of the total protein in the extract. In the BL21(DE3)/pFS766 IPTG-induced culture more than 50% of the DHDPS protein was in the pellet fraction. The pellet fractions in both cases were 90-95% pure DHDPS, with no other single protein present in significant amounts. Thus, these fractions were pure enough for use in the generation of antibodies. The pellet fractions containing 2-4 mg of either *E. coli* DHDPS or *Corynebacterium* DHDPS were solubilized in 50 mM NaCl; 50 mM Tris-Cl, pH 7.5; 1 mM EDTA, 0.2 mM dithiothreitol, 0.2% SDS and sent to Hazelton Research Facility (310 Swampridge Road, Denver, PA 17517) to have rabbit antibodies raised against the proteins.

DHDPS enzyme activity was assayed as follows:
Assay mix (for 10 X 1.0 mL assay tubes or 40 X 0.25 mL for microtiter dish); made fresh, just before use:

2.5 mL	H_20
0.5 mL	1.0 M Tris-HCl pH8.0
0.5 mL	0.1 M Na Pyruvate
0.5 mL	o-Aminobenzaldehyde (10mg/mL in ethanol)
25 µL	1.0M DL-Aspartic-β-semialdehyde (ASA) in 1.0N HCl

	Assay (1.0 mL):	MicroAssay (0.25mL):
DHDPS assay mix	0.40 mL	0.10 mL
enzyme extract + H_20;	0.10 mL	.025 mL
10 mM L-lysine	5 µL or 20 µL	1 µL or 5 µL

Incubate at 30° for desired time. Stop by addition of:

1.0 N HCl	0.50 mL	0.125 mL

Color allowed to develop for 30-60 min. Precipitate spun down in eppendorf centrifuge. OD_{540} vs 0 min read as blank. For MicroAssay, aliquot 0.2 mL into microtiter well and read at OD_{530}.

The specific activity of *E. coli* DHDPS in the supernatant fraction of induced extracts was about 50 OD_{540} units per minute per milligram protein in a 1.0 mL assay. *E. coli* DHDPS was sensitive to the presence of L-lysine in the assay. Fifty percent inhibition was found at a concentration of about 0.5 mM. For *Corynebacterium* DHDPS, the activity was measured in the supernatant fraction of uninduced extracts, rather than induced extracts. Enzyme activity was about 4 OD_{530} units per min per milligram protein in a 0.25 mL assay. In contrast to *E. coli* DHDPS, *Corynebacterium* DHDPS was not inhibited at all by L-lysine, even at a concentration of 70 mM.

EXAMPLE 5

Excretion of amino acids by *E. coli* expressing high levels of DHDPS and/or AKIII

The *E. coli* expression cassette with the *E. coli* dapA gene linked to the T7 RNA polymerase promoter was isolated by digesting pBT442 (see Example 4) with Bgl II and BamH I separating the digestion products via agarose gel electrophoresis and eluting the approximately 1250 bp fragment from the gel. This fragment was inserted into the BamH I site of plasmids pBT461 (containing the T7 promoter/lysC gene) and pBT492 (containing the T7 promoter/lysC-M4 gene). Inserts where transcription of both genes would be in the same direction were identified by restriction endonuclease analysis yielding plasmids pBT517 (T7/dapA + T7/lysC-M4) and pBT519 (T7/dapA + T7/lysC).

In order to induce *E. coli* to produce and excrete amino acids, these plasmids, as well as plasmids pBT442, pBT461 and pBT492 (and pBR322 as a control) were transformed into *E. coli* strain BL21(DE3) [Studier et al. (1986) *J. Mol. Biol. 189*:113-130]. All of these plasmids, but especially pBT517 and pBT519, are somewhat unstable in this host strain, necessitating careful maintenance of selection for ampicillin resistance during growth.

All strains were grown in minimal salts M9 media [see Sambrook et al. (1989) Molecular Cloning, *A Laboratory Manual,* Cold Spring Harbor Laboratory Press] supplemented with ampicillin to maintain selection for the plasmids overnight at 37°. Cultures were collected when they reached an OD_{600} of 1. Cells were removed by centrifugation and the supernatants (3 mL) were passed through 0.2 micron filters to remove remaining cells and large molecules. Five microliter aliquots of the supernatant fractions were analyzed for amino acid composition with a Beckman Model 6300 amino acid analyzer using post-column ninhydrin detection. Results are shown in Table 1.

TABLE 1

Amino Acid Concentration in Culture Supernatants [mM]

Plasmid	Lys	Thr	Met	Ala	Val	Asp	Glu
pBR322	0	0	0	0.05	0.1	0	0
pBT442	0.48	0	0	0.04	0.06	0	0
pBT461	0.14	0.05	0	0.02	0.03	0	0
pBT492	0.16	0.07	0	0.02	0.03	0	0
pBT517	0.18	0	0.01	0	0	0.02	0.02
pBT519	0.14	0	0.01	0	0	0.01	0

All of the plasmids, except the pBR322 control, lead to the excretion of lysine into the culture medium. Expression of the lysC or the lysC-M4 gene lead to both lysine and threonine excretion. Expression of lysC-M4 + dapA lead to excretion of lysine, methionine, aspartic acid and glutamic acid, but not threonine. In addition, alanine and valine were not detected in the culture supernatant. Similar results were obtained with lysC + dapA, except that no glutamic acid was excreted.

EXAMPLE 6

Construction of Chimeric dapA, lysC and lysC-M4 Genes for Expression in Plants

Several gene expression cassettes were used for construction of chimeric genes for expression of ecodapA, cordapA, lysC and lysC-M4 in plants. A leaf expression cassette (Figure 4a) is composed of the 35S promoter of cauliflower mosaic virus [Odell et al.(1985) *Nature 313*:810-812; Hull et al. (1987) *Virology 86*:482-493], the translation leader from the chlorophyll a/b binding protein (Cab) gene, [Dunsmuir (1985) *Nucleic Acids Res. 13*:2503-2518] and 3' transcription termination region from the nopaline synthase (Nos) gene [Depicker et al. (1982) *J. Mol. Appl. Genet. 1*:561-570]. Between the 5' and 3' regions are the restriction endonuclease sites Nco I (which includes the ATG translation initiation codon), EcoR I, Sma I and Kpn I. The entire cassette is flanked by Sal I sites; there is also a BamH I site upstream of the cassette.

A seed-specific expression cassette (Figure 4b) is composed of the promoter and transcription terminator from the gene encoding the β subunit of the seed storage protein phaseolin from the bean *Phaseolus vulgaris* [Doyle et al. (1986) *J. Biol. Chem. 261*:9228-9238]. The phaseolin cassette includes about 500 nucleotides upstream (5') from the translation initiation codon and about 1650 nucleotides downstream (3') from the translation stop codon of phaseolin. Between the 5' and 3' regions are the unique restriction endonuclease sites Nco I

(which includes the ATG translation initiation codon), Sma I, Kpn I and Xba I. The entire cassette is flanked by Hind III sites.

A second seed expression cassette was used for the cordapA gene. This was composed of the promoter and transcription terminator from the soybean Kunitz tyrosine inhibitor 3 (KTI3) gene [Jofuku et al. (1989) *Plant Cell 1*:427-435]. The KTI3 cassette includes about 2000 nucleotides upstream (5') from the translation initiation codon and about 240 nucleotides downstream (3') from the translation stop codon of phaseolin. Between the 5' and 3' regions are the unique restriction endonuclease sites Nco I (which includes the ATG translation initiation codon), Xba I, Kpn I and Sma I. The entire cassette is flanked by BamH I sites.

A constitutive expression cassette for corn was used for expression of the lysC-M4 gene and the ecodapA gene. It was composed of a chimeric promoter derived from pieces of two corn promoters and modified by *in vitro* site-specific mutagenesis to yield a high level constitutive promoter and a 3' region from a corn gene of unknown function. Between the 5' and 3' regions are the unique restriction endonuclease sites Nco I (which includes the ATG translation initiation codon), Sma I and Bgl II. The nucleotide sequence of the constitutive corn expression cassette is shown in SEQ ID NO:93.

Plant amino acid biosynthetic enzymes are known to be localized in the chloroplasts and therefore are synthesized with a chloroplast targeting signal. Bacterial proteins such as DHDPS and AKIII have no such signal. A chloroplast transit sequence (cts) was therefore fused to the ecodapA, cordapA, lysC, and lysC-M4 coding sequence in some chimeric genes. The cts used was based on the cts of the small subunit of ribulose 1,5-bisphosphate carboxylase from soybean [Berry-Lowe et al. (1982) *J. Mol. Appl. Genet. 1*:483-498]. The oligonucleotides SEQ ID NOS:8-11 were synthesized and used as described below. For corn the cts used was based on the cts of the small subunit of ribulose 1,5-bisphosphate carboxylase from corn [Lebrun et al. (1987) *Nucleic Acids Res. 15*:4360] and is designated mcts to distinguish it from the soybean cts. The oligonucleotides SEQ ID NOS:17-22 were synthesized and used as described below.

Fourteen chimeric genes were created:

No. 1) 35S promoter/Cab leader/lysC/Nos 3'

No. 2) 35S promoter/Cab leader/cts/lysC/Nos 3'

No. 3) 35S promoter/Cab leader/cts/lysC-M4/Nos 3'

No. 4) phaseolin 5' region/cts/lysC/phaseolin 3' region

No. 5) phaseolin 5' region/cts/lysC-M4/phaseolin 3' region

No. 6) 35S promoter/Cab leader/ecodapA/Nos 3'

No. 7) 35S promoter/Cab leader/cts/ecodapA/Nos 3

No. 8) phaseolin 5' region/ecodapA/phaseolin 3' region

No. 9) phaseolin 5' region/cts/ecodapA/phaseolin 3' region

No. 10) 35S promoter/Cab leader/cts/cordapA/Nos 3

No. 11) phaseolin 5' region/cts/cordapA/phaseolin 3' region

No. 12) KTI3 5' region/cts/cordapA/KTI3 3' region

No. 13) HH534 5' region/mcts/lysC-M4/HH2-1 3' region

No. 14) HH534 5' region/mcts/ecodapA/HH2-1 3' region

A 1440 bp Nco I-Hpa I fragment containing the entire lysC coding region plus about 90 bp of 3' non-coding sequence was isolated from an agarose gel following electrophoresis and inserted into the leaf expression cassette digested with Nco I and Sma I (chimeric gene No. 1), yielding plasmid pBT483.

Oligonucleotides SEQ ID NO:8 and SEQ ID NO:9, which encode the carboxy terminal part of the chloroplast targeting signal, were annealed, resulting in Nco I compatible ends, purified via polyacrylamide gel electrophoresis, and inserted into Nco I digested pBT461. The insertion of the correct sequence in the correct orientation was verified by DNA sequencing yielding pBT496. Oligonucleotides SEQ ID NO:10 and SEQ ID NO:11, which encode the amino terminal part of the chloroplast targeting signal, were annealed, resulting in Nco I compatible ends, purified via polyacrylamide gel electrophoresis, and inserted into Nco I digested pBT496. The insertion of the correct sequence in the correct orientation was verified by DNA sequencing yielding pBT521. Thus the cts was fused to the lysC gene.

To fuse the cts to the lysC-M4 gene, pBT521 was digested with Sal I, and an approximately 900 bp DNA fragment that included the cts and the amino terminal coding region of lysC was isolated. This fragment was inserted into Sal I digested pBT492, effectively replacing the amino terminal coding region of lysC-M4 with the fused cts and the amino terminal coding region of lysC. Since the mutation that resulted in lysine-insensitivity was not in the replaced fragment, the new plasmid, pBT523, carried the cts fused to lysC-M4.

The 1600 bp Nco I-Hpa I fragment containing the cts fused to lysC plus about 90 bp of 3' non-coding sequence was isolated and inserted into the leaf expression cassette digested with Nco I and Sma I (chimeric gene No. 2), yielding plasmid pBT541 and the seed-specific expression cassette digested with Nco I and Sma I (chimeric gene No. 4), yielding plasmid pBT543.

Similarly, the 1600 bp Nco I-Hpa I fragment containing the cts fused to lysC-M4 plus about 90 bp of 3' non-coding sequence was isolated and inserted into the leaf expression cassette digested with Nco I and Sma I (chimeric gene No. 3), yielding plasmid pBT540 and the seed-specific expression cassette digested with Nco I and Sma I (chimeric gene No. 5), yielding plasmid pBT544.

Before insertion into the expression cassettes, the ecodapA gene was modified to insert a restriction endonuclease site, Kpn I, just after the translation stop codon. The oligonucleotides SEQ ID NOS:12-13 were synthesized for this purpose:

SEQ ID NO:12:
CCGGTTTGCT GTAATAGGTA CCA

SEQ ID NO:13:
AGCTTGGTAC CTATTACAGC AAACCGGCAT G

Oligonucleotides SEQ ID NO:12 and SEQ ID NO:13 were annealed, resulting in an Sph I compatible end on one end and a Hind III compatible end on the other and inserted into Sph I plus Hind III digested pBT437. The insertion of the correct sequence was verified by DNA sequencing yielding pBT443.

An 880 bp Nco I-Kpn I fragment from pBT443 containing the entire ecodapA coding region was isolated from an agarose gel following electrophoresis and inserted into the leaf expression cassette digested with Nco I and Kpn I (chimeric gene No. 6), yielding plasmid pBT450 and into the seed-specific expression cassette digested with Nco I and Kpn I (chimeric gene No. 8), yielding plasmid pBT494.

Oligonucleotides SEQ ID NO:8 and SEQ ID NO:9, which encode the carboxy terminal part of the chloroplast targeting signal, were annealed resulting in Nco I compatible ends, purified via polyacrylamide gel electrophoresis, and inserted into Nco I digested pBT450. The insertion of the correct sequence in the correct orientation was verified by DNA sequencing yielding pBT451. A 950 bp Nco I-Kpn I fragment from pBT451 encoding the carboxy terminal part of the chloroplast targeting signal fused to the entire ecodapA coding region was isolated from an agarose gel following electrophoresis and inserted into the seed-specific expression cassette digested with Nco I and Kpn I, yielding plasmid pBT495. Oligonucleotides SEQ ID NO:10: and SEQ ID NO:11:, which encode the amino terminal part of the chloroplast targeting signal, were annealed resulting in Nco I compatible ends, purified via polyacrylamide gel electrophoresis, and inserted into Nco I digested pBT451 and pBT495. Insertion of the correct sequence in the correct orientation was verified by DNA sequencing yielding pBT455 and pBT520, respectively. Thus the cts was fused to the ecodapA gene in the leaf expression cassette (chimeric gene No. 7) and the seed-specific expression cassette (chimeric gene No. 9).

An 870 bp Nco I-EcoR I fragment from pFS766 containing the entire cordapA coding region was isolated from an agarose gel following electrophoresis and inserted into the leaf expression cassette digested with Nco I and EcoR I, yielding plasmid pFS789. To attach the cts to the cordapA gene, a DNA fragment containing the entire cts was prepared using PCR. The template DNA was pBT540 and the oligonucleotide primers used were:

SEQ ID NO:14:
GCTTCCTCAA TGATCTCCTC CCCAGCT

SEQ ID NO:15:
CATTGTACTC TTCCACCGTT GCTAGCAA

PCR was performed using a Perkin-Elmer Cetus kit according to the instructions of the vendor on a thermocycler manufactured by the same company. The PCR-generated 160 bp fragment was treated with T4 DNA polymerase in the presence of the 4 deoxyribonucleotide triphosphates to obtain a blunt-ended fragment. The cts fragment was inserted into pFS789 which had been digested with Nco I and treated with the Klenow fragment of DNA polymerase to fill in the 5' overhangs. The inserted fragment and the vector/insert junctions were determined to be correct by DNA sequencing, yielding pFS846 containing chimeric gene No. 10.

A 1030 bp Nco I-Kpn I fragment from pFS846 containing the cts attached to the cordapA coding region was isolated from an agarose gel following electrophoresis and inserted into the phaseolin seed expression cassette digested with Nco I and Kpn I, yielding plasmid pFS889 containing chimeric gene No. 11. Similarly, the 1030 bp Nco I-Kpn I fragment from pFS846 was inserted into the KTI3 seed expression cassette digested with Nco I and Kpn I, yielding plasmid pFS862 containing chimeric gene No. 12.

Oligonucleotides SEQ ID NO:94 and SEQ ID NO:95, which encode the carboxy terminal part of the corn chloroplast targeting signal, were annealed, resulting in Xba I and Nco I compatible ends, purified via polyacrylamide gel electrophoresis, and inserted into Xba I plus Nco I digested pBT492 (see Example 2). The insertion of the correct sequence was verified by DNA sequencing yielding pBT556. Oligonucleotides SEQ ID NO:96 and SEQ ID NO:97, which encode the middle part of the chloroplast targeting signal, were annealed, resulting in Bgl II and Xba I compatible ends, purified via polyacrylamide gel electrophoresis, and inserted into Bgl II and Xba I digested pBT556. The

insertion of the correct sequence was verified by DNA sequencing yielding pBT557. Oligonucleotides SEQ ID NO:98 and SEQ ID NO:99, which encode the amino terminal part of the chloroplast targeting signal, were annealed, resulting in Nco I and Afl II compatible ends, purified via polyacrylamide gel electrophoresis, and inserted into Nco I and Afl II digested pBT557. The insertion of the correct sequence was verified by DNA sequencing yielding pBT558. Thus the mcts was fused to the lysC-M4 gene.

A 1.6 kb Nco I-Hpa I fragment from pBT558 containing the mcts attached to the lysC-M4 gene was isolated from an agarose gel following electrophoresis and inserted into the constitutive corn expression cassette digested with Nco I and Sma I, yielding plasmid pBT573 containing chimeric gene No. 13.

To attach the mcts to the ecodapA gene a DNA fragment containing the entire mcts was prepared using PCR as described above. The template DNA was pBT558 and the oligonucleotide primers used were:

SEQ ID NO:100:
GCGCCCACCG TGATGA

SEQ ID NO:101:
CACCGGATTC TTCCGC

The mcts fragment was inserted into pBT450 (above) which had been digested with Nco I and treated with the Klenow fragment of DNA polymerase to fill in the 5' overhangs. The inserted fragment and the vector/insert junctions were determined to be correct by DNA sequencing, yielding pBT576. Plasmid pBT576 was digested with Asp718, treated with the Klenow fragment of DNA polymerase to yield a blunt-ended fragment, and then digested with Nco I. The resulting 1030 bp Nco I-blunt-ended fragment containing the ecodapA gene attached to the mcts was isolated from an agarose gel following electrophoresis. This fragment was inserted into the constitutive corn expression cassette digested with Bgl II, treated with the Klenow fragment of DNA polymerase to yield a blunt-ended fragment, and then digested with Nco I, yielding plasmid pBT583 containing chimeric gene No. 14.

EXAMPLE 7

Transformation of Tobacco with the 35S Promoter/lysC Chimeric Genes

Transformation of tobacco with the 35S promoter/lysC chimeric genes was effected according to the following:

The 35S promoter/Cab leader/lysC/Nos 3', 35S promoter/Cab leader/cts/lysC/Nos 3', and 35S promoter/Cab leader/cts/lysC-M4/Nos 3' chimeric genes were isolated as 3.5-3.6 kb BamH I-EcoR I fragments and inserted into BamH I-EcoR I digested vector pZS97K (Figure 5), yielding plasmids pBT497, pBT545 and pBT542, respectively. The vector is part of a binary Ti plasmid vector system [Bevan, (1984) *Nucl. Acids. Res. 12*:8711-8720] of *Agrobacterium tumefaciens*. The vector contains: (1) the chimeric gene nopaline synthase promoter/neomycin phosphotransferase coding region (nos:NPT II) as a selectable marker for transformed plant cells [Bevan et al. (1983) *Nature 304*:184-186]; (2) the left and right borders of the T-DNA of the Ti plasmid [Bevan (1984) *Nucl. Acids. Res. 12*:8711-8720]; (3) the *E. coli* lacZ α-complementing segment [Viera and Messing (1982) *Gene 19*:259-267] with unique restriction endonuclease sites for EcoR I, Kpn I, BamH I and Sal I; (4) the bacterial replication origin from the *Pseudomonas* plasmid pVS1 [Itoh et al. (1984) *Plasmid 11*:206-220]; and (5) the bacterial neomycin phosphotransferase gene from Tn5 [Berg et al. (1975) *Proc. Natl. Acad Sci. U.S.A. 72*:3628-3632] as a selectable marker for transformed *A. tumefaciens*.

The 35S promoter/Cab leader/cts/lysC/Nos 3', and 35S promoter/Cab leader/cts/lysC-M4/Nos 3' chimeric genes were also inserted into the binary vector pBT456, yielding pBT547 and pBT546, respectively. This vector is pZS97K, into which the chimeric gene 35S promoter/Cab leader/cts/dapA/Nos 3' had previously been inserted as a BamH I-Sal I fragment (see Example 9). In the cloning process large deletions of the dapA chimeric gene occurred. As a consequence these plasmids are equivalent to pBT545 and pBT542, in that the only transgene expressed in plants (other than the selectable marker gene, NPT II) was 35S promoter/Cab leader/cts/lysC/Nos 3' or 35S promoter/Cab leader/cts/lysC-M4/Nos 3'.

The binary vectors containing the chimeric lysC genes were transferred by tri-parental matings [Ruvkin et al. (1981) Nature 289:85-88] to *Agrobacterium* strain LBA4404/pAL4404 [Hockema et al (1983), *Nature 303*:179-180]. The *Agrobacterium* transformants were used to inoculate tobacco leaf disks [Horsch et al. (1985) *Science 227*:1229-1231]. Transgenic plants were regenerated in selective medium containing kanamycin.

To assay for expression of the chimeric genes in leaves of the transformed plants, protein was extracted as follows. Approximately 2.5 g of young plant leaves, with the midrib removed, were placed in a dounce homogenizer with 0.2 g of polyvinyl polypyrrolidone and 11 mL of 50mM Tris-HCl pH8.0, 50mM NaCl, 1mM EDTA (TNE) and ground thoroughly. The suspension was further

homogenized by a 20 sec treatment with a Brinkman Polytron Homogenizer operated at setting 7. The resultant suspensions were centrifuged at 16,000 rpm for 20 min at 4° in a Dupont-Sorvall superspeed centrifuge using an SS34 rotor to remove particulates. The supernatant was decanted, the volume was adjusted to be 10 mL by addition of TNE if necessary, and 8 mL of cold, saturated ammonium sulfate was added. The mixture was set on ice for 30 min and centrifuged as described above. The supernatant was decanted and the pellet, which contained the AKIII protein, was resuspended in 1 mL of TNE and desalted by passage over a Sephadex G-25 M column (Column PD-10, Pharmacia).

For immunological characterization, three volumes of extract were mixed with 1 volume of 4 X SDS-gel sample buffer (0.17M Tris-HCl pH6.8, 6.7% SDS, 16.7% (v/v) β-mercaptoethanol, 33% (v/v) glycerol) and 3 μL from each extract were run per lane on an SDS polyacrylamide gel, with bacterially produced AKIII serving as a size standard and protein extracted from untransformed tobacco leaves serving as a negative control. The proteins were then electrophoretically blotted onto a nitrocellulose membrane (Western Blot). The membranes were exposed to the AKIII antibodies prepared as described in Example 2 at a 1:5000 dilution of the rabbit serum using standard protocol provided by BioRad with their Immun-Blot Kit. Following rinsing to remove unbound primary antibody, the membranes were exposed to the secondary antibody, donkey anti-rabbit Ig conjugated to horseradish peroxidase (Amersham) at a 1:3000 dilution. Following rinsing to remove unbound secondary antibody, the membranes were exposed to Amersham chemiluminescence reagent and X-ray film.

Seven of thirteen transformants containing the chimeric gene, 35S promoter/Cab leader/cts/lysC-M4/Nos 3', and thirteen of seventeen transformants containing the chimeric gene, 35S promoter/Cab leader/cts/lysC/Nos 3', produced AKIII protein (Table 2). In all cases protein which reacted with the AKIII antibody was of several sizes. Approximately equal quantities of proteins equal in size to AKIII produced in E. coli, and a protein about 6 kd larger were evident in all samples, suggesting that the chloroplast targeting signal had been removed from about half of the protein synthesized. This further suggests that about half of the protein entered the chloroplast. In addition, a considerable amount of protein of higher molecular weight was observed. The origin of this protein is unclear; the total amount present was equal or slightly greater than the amounts of the mature and putative AKIII precursor proteins combined.

The leaf extracts were assayed for AK activity as described in Example 2. AKIII could be distinguished from endogenous AK activity, if it were present, by its increased resistance to lysine plus threonine. Unfortunately, however, this

assay was not sensitive enough to reliably detect AKIII activity in these extracts. Zero of four transformants containing the chimeric gene, 35S promoter/Cab leader/lysC/Nos 3', showed AKIII activity. Only one extract, from a transformant containing the 35S promoter/Cab leader/cts/lysC-M4/Nos 3' gene, produced a convincing level of enzyme activity. This came from transformant 546-49A, and was also the extract that showed the highest level of AKIII-M4 protein via Western blot.

An alternative method to detect the expression of active AKIII enzyme was to evaluate the sensitivity or resistance of leaf tissue to high concentrations of lysine plus threonine. Growth of cell cultures and seedlings of many plants is inhibited by high concentrations of lysine plus threonine; this is reversed by addition of methionine (or homoserine which is converted to methionine in vivo). Lysine plus threonine inhibition is thought to result from feedback inhibition of endogenous AK, which reduces flux through the pathway leading to starvation for methionine. In tobacco there are two AK enzymes in leaves, one lysine-sensitive and one threonine sensitive [Negrutui et al. (1984) Theor. Appl. Genet. 68:11-20]. High concentrations of lysine plus threonine inhibit growth of shoots from tobacco leaf disks and inhibition is reversed by addition of low concentrations of methionine. Thus, growth inhibition is presumably due to inhibition of the two AK isozymes.

Expression of active lysine and threonine insensitive AKIII-M4 would be predicted to reverse the growth inhibition. As can be seen in Table 2, this was observed. There is, in fact, a good correlation between the level of AKIII-M4 protein expressed and the resistance to lysine plus threonine inhibition. Expression of lysine-sensitive wild type AKIII does not have a similar effect. Only the highest expressing transformant showed any resistance to lysine plus threonine inhibition, and this was much less dramatic than that observed with AKIII-M4.

To measure free amino acid composition of the leaves, free amino acids were extracted as follows. Approximately 30-40 mg of young leaf tissue was chopped with a razor and dropped into 0.6 mL of methanol/ chloroform/water mixed in ratio of 12v/5v/3v (MCW) on dry ice. After 10-30 min the suspensions were brought to room temperature and homogenized with an Omni 1000 Handheld Rechargeable Homogenizer and then centrifuged in an eppendorf microcentrifuge for 3 min. Approximately 0.6 mL of supernatant was decanted and an additional 0.2 mL of MCW was added to the pellet which was then vortexed and centrifuged as above. The second supernatant, about 0.2 mL, was added to the first. To this, 0.2mL of chloroform was added followed by 0.3 mL of

water. The mixture was vortexed and the centrifuged in an eppendorf microcentrifuge for about 3 min, the upper aqueous phase, approximately 1.0 mL, was removed, and was dried down in a Savant Speed Vac Concentrator. One-tenth of the sample was run on a Beckman Model 6300 amino acid analyzer using post-column ninhydrin detection. Relative free amino acid levels in the leaves were compared as ratios of lysine or threonine to leucine, thus using leucine as an internal standard. There was no consistent effect of expression of AKIII or AKIII-M4 on the lysine or threonine (or any other amino acid) levels in the leaves (Table 2).

TABLE 2

BT542 transformants: 35S promoter/Cab leader/cts/lysC-M4/Nos 3'
BT545 transformants: 35S promoter/Cab leader/cts/lysC/Nos 3'
BT546 transformants: 35S promoter/Cab leader/cts/lysC-M4/Nos 3'
BT547 transformants: 35S promoter/Cab leader/cts/lysC/Nos 3'

LINE	FREE AMINO ACIDS/LEAF K/L	T/L	AKIII ACTIVITY U/MG/HR	WESTERN BLOT	RESISTANCE TO Lys 3mM + Thr 3mM
542-5B	0.5	3.5	0	-	-
542-26A	0.5	3.3	0	-	-
542-27B	0.5	3.4	0	++	+++
542-35A	0.5	4.3	0.01	-	-
542-54A	0.5	2.8	0	-	-
542-57B	O.5	3.4	0	-	+
545-5A	n.d.	n.d.	0.02	++	
545-7B	0.5	3.4	0	+	
545-17B	0.6	2.5	0.01	+	
545-27A	0.6	3.5	0	++	
545-50E	0.6	3.6	0.03	++	
545-52A	0.5	3.6	0.02	-	
546-4A	0.4	4.5	0	+	+
546-24B	0.6	4.9	0.04	++	++
546-44A	0.5	6.0	0.03	+	++
546-49A	0.7	7.0	0.10	+++	+++
546-54A	0.5	6.4	0	+	+
546-56B	0.5	4.4	0.01	-	-
546-58B	0.6	8.0	0	+	++
547-3D	0.4	5.4	0	++	-
547-8B	0.6	5.0	0.02	-	

547-9A	0.5	4.3	0.03	+++	
547-12A	0.7	3.9	0	+++	+
547-15B	0.6	4.5	0	+	-
547-16A	0.5	3.6	0	++	
547-18A	0.5	4.0		+++	-
547-22A	0.8	4.4		-	
547-25C	0.5	4.3		+	-
547-28C	0.6	5.6		-	
547-29C	0.5	3.8		+++	+

EXAMPLE 8

Transformation of Tobacco with the Phaseolin Promoter/lysC Chimeric Genes

The phaseolin promoter/lysC chimeric gene cassettes, phaseolin 5' region/cts/lysC/phaseolin 3' region, and phaseolin 5' region/cts/lysC-M4/phaseolin 3' region (Example 6) were isolated as approximately 3.3 kb Hind III fragments. These fragments were inserted into the unique Hind III site of the binary vector pZS97 (Figure 6) yielding pBT548 and pBT549, respectively. This vector is similar to pZS97K described in Example 7 except for the presence of two additional unique cloning sites, Sma I and Hind III, and the bacterial β-lactamase gene (causing ampicillin resistance) as a selectable marker for transformed *A. tumefaciens* instead of the bacterial neomycin phosphotransferase gene.

The binary vectors containing the chimeric lysC genes were transferred by tri-parental matings to *Agrobacterium* strain LBA4404/pAL4404, the *Agrobacterium* transformants were used to inoculate tobacco leaf disks and transgenic plants regenerated by the methods set out in Example 7.

To assay for expression of the chimeric genes in the seeds of the transformed plants, the plants were allowed to flower, self-pollinate and go to seed. Total proteins were extracted from mature seeds as follows. Approximately 30-40 mg of seeds were put into a 1.5mL disposable plastic microfuge tube and ground in 0.25 mL of 50 mM Tris-HCl pH6.8, 2 mM EDTA, 1% SDS, 1% (v/v) β-mercaptoethanol. The grinding was done using a motorized grinder with disposable plastic shafts designed to fit into the microfuge tube. The resultant suspensions were centrifuged for 5 min at room temperature in a microfuge to remove particulates. Three volumes of extract was mixed with 1 volume of 4 X SDS-gel sample buffer (0.17 M Tris-HCl pH 6.8, 6.7% SDS, 16.7% (v/v) β-mercaptoethanol, 33% (v/v) glycerol) and 5 μL from each extract were run per lane on an SDS polyacrylamide gel, with bacterially produced AKIII serving as a size standard and protein extracted from untransformed tobacco seeds serving as a

negative control. The proteins were then electrophoretically blotted onto a nitrocellulose membrane. The membranes were exposed to the AKIII antibodies (prepared as described in Example 2) at a 1:5000 dilution of the rabbit serum using standard protocol provided by BioRad with their Immun-Blot Kit. Following rinsing to remove unbound primary antibody the membranes were exposed to the secondary antibody, donkey anti-rabbit Ig conjugated to horseradish peroxidase (Amersham) at a 1:3000 dilution. Following rinsing to remove unbound secondary antibody, the membranes were exposed to Amersham chemiluminescence reagent and X-ray film.

Ten of eleven transformants containing the chimeric gene, phaseolin 5' region/cts/lysC/phaseolin 3' region, and ten of eleven transformants containing the chimeric gene, phaseolin 5' region/cts/lysC-M4/phaseolin 3' region, produced AKIII protein (Table 3). In all cases protein which reacted with the AKIII antibody was of several sizes. Approximately equal quantities of proteins equal in size to AKIII produced in E. coli, and about 6 kd larger were evident in all samples, suggesting that the chloroplast targeting signal had been removed from about half of the protein synthesized. This further suggests that about half of the protein entered the chloroplast. In addition, some proteins of lower molecular weight were observed, probably representing breakdown products of the AKIII polypeptide.

To measure free amino acid composition of the seeds, free amino acids were extracted from mature seeds as follows. Approximately 30-40 mg of seeds and an approximately equal amount of sterilized sand were put into a 1.5 mL disposable plastic microfuge tube along with 0.2 mL of methanol/chloroform/water mixed in ratio of 12v/5v/3v (MCW) at room temperature. The seeds were ground using a motorized grinder with disposable plastic shafts designed to fit into the microfuge tube. After grinding an additional 0.5 mL of MCW was added, the mixture was vortexed and then centrifuged in an eppendorf microcentrifuge for about 3 min. Approximately 0.6 mL of supernatant was decanted and an additional 0.2 mL of MCW was added to the pellet which was then vortexed and centrifuged as above. The second supernatant, about 0.2 mL, was added to the first. To this, 0.2 mL of chloroform was added followed by 0.3 mL of water. The mixture was vortexed and then centrifuged in an eppendorf microcentrifuge for about 3 min, the upper aqueous phase, approximately 1.0 mL, was removed, and was dried down in a Savant Speed Vac Concentrator. The samples were hydrolyzed in 6N hydrochloric acid, 0.4% (v/v) β-mercaptoethanol under nitrogen for 24 h at 110-120°; 1/4 of the sample was run on a Beckman Model 6300 amino acid analyzer using post-column ninhydrin detection. Relative free amino acid levels

in the seeds were compared as ratios of lysine, methionine, threonine or isoleucine to leucine, thus using leucine as an internal standard.

To measure the total amino acid composition of the seeds, 6 seeds were hydrolyzed in 6 N hydrochloric acid, 0.4% (v/v) β-mercaptoethanol under nitrogen for 24 h at 110-120°; 1/10 of the sample was run on a Beckman Model 6300 amino acid analyzer using post-column ninhydrin detection. Relative amino acid levels in the seeds were compared as ratios of lysine, methionine, threonine or isoleucine to leucine, thus using leucine as an internal standard. Because the transgene was segregating in these self-pollinated progeny of the primary transformant and only six seeds were analyzed, there was expected to be some sampling error. Therefore, the measurement was repeated multiple times for some of the lines (Table 3).

Expression of the cts/lysC gene in the seeds resulted in a 2 to 4-fold increase in the level of free threonine in the seeds and a 2 to 3-fold increase in the level of free lysine in some cases. There was a good correlation between transformants expressing higher levels of AKIII protein and those having higher levels of free threonine, but this was not the case for lysine. These relatively small increases of free threonine or lysine were not sufficient to yield detectable increases in the levels of total threonine or lysine in the seeds. Expression of the cts/lysC-M4 gene in the seeds resulted in a 4 to 23-fold increase in the level of free threonine in the seeds and a 2 to 3-fold increase in the level of free lysine in some cases. There was a good correlation between transformants expressing higher levels of AKIII protein and those having higher levels of free threonine, but this was again not the case for lysine. The larger increases of free threonine were sufficient to yield detectable increases in the levels of total threonine in the seeds. Sixteen to twenty-five percent increases in total threonine content of the seeds were observed in three lines which were sampled multiple times. (Isoleucine to leucine ratios are shown for comparison.) The lines that showed increased total threonine were the same ones the showed the highest levels of increase in free threonine and high expression of the AKIII-M4 protein. From these results it can be estimated that free threonine represents about 1% of the total threonine present in a normal tobacco seed, but about 18% of the total threonine present in seeds expressing high levels of AKIII-M4.

TABLE 3

BT548 Transformants: phaseolin 5' region/cts/lysC/phaseolin 3'
BT549 Transformants: phaseolin 5' region/cts/lysC-M4/phaseolin 3'

	SEED FREE AMINO ACID			SEED TOTAL AMINO ACID			
LINE	K/L	T/L	I/L	K/L	T/L	I/L	WESTERN

NORMAL	0.49	1.34	0.68	0.35	0.68	0.63	-
548-2A	1.15	2.3	0.78	0.43	0.71	0.67	+
548-4D	0.69	5.3	0.80	0.35	0.69	0.65	+++
548-6A	0.39	3.5	0.85	0.35	0.69	0.64	+
548-7A	0.82	4.2	0.83	0.36	0.68	0.65	++
548-14A	0.41	3.1	0.82	0.32	0.67	0.65	+
548-18A	0.51	1.5	0.69	0.37	0.67	0.63	-
548-22A	1.41	2.9	0.75	0.47	0.74	0.65	+++
548-24A	0.73	3.7	0.81	0.38	0.68	0.65	++
548-41A	0.40	2.8	0.77	0.37	0.68	0.65	+
548-50A	0.46	4.0	0.81	0.33	0.68	0.65	+
548-57A	0.50	3.8	0.80	0.33	0.67	0.65	++
549-5A	0.63	5.9	0.69	0.32	0.65	0.65	+
549-7A	0.51	8.3	0.78	0.33	0.67	0.63	++
549-20A	0.67	30	0.88	0.38*	0.82*	0.65*	++++
549-34A	0.43	1.3	0.69	0.32	0.64	0.63	-
549-39D	0.83	16	0.83	0.35	0.71	0.63	+++
549-40A	0.80	4.9	0.74	0.33	0.63	0.64	+
549-41C	0.99	13	0.80	0.38*	0.79*	0.65*	+++
549-46A	0.48	7.7	0.84	0.34	0.70	0.64	+
549-52A	0.81	9.2	0.80	0.39	0.70	0.65	++
549-57A	0.60	15	0.77	0.35*	0.85*	0.64*	+++
549-60D	0.85	11	0.79	0.37	0.73	0.65	++

Normal was calculated as the average of 6 samples for free amino acid and 23 samples for total amino acids.

* Indicates average of at least 5 samples

Seeds derived from self-pollination of two plants transformed with the phaseolin 5' region/cts/lysC-M4/phaseolin 3' region, plants 549-5A and 549-40A, showed 3 kanamycin resistant to 1 kanamycin sensitive seedlings, indicative of a single site of insertion of the transgene. Progeny plants were grown, self-pollinated and seed was analyzed for segregation of the kanamycin marker gene. Progeny plants that were homozygous for the transgene insert, thus containing two copies of the gene cassette, accumulated approximately 2 times as much threonine in their seed as their sibling heterozygous progeny with one copy of the gene cassette and about 8 times as much as seed without the gene. This demonstrates that the level of expression of the E. coli enzyme controls the accumulation of free threonine.

EXAMPLE 9

Transformation of Tobacco with the 35S Promoter/ecodapA Chimeric Genes

The 35S promoter/Cab leader/ecodapA/Nos 3' and 35S promoter/Cab leader/cts/ecodapA/Nos 3', chimeric genes were isolated as 3.1, and 3.3 kb BamH I-Sal I fragments, respectively and inserted into BamH I-Sal I digested binary vector pZS97K (Figure 5), yielding plasmids pBT462 and pBT463, respectively. The binary vector is described in Example 7.

The binary vectors containing the chimeric ecodapA genes were transferred by tri-parental matings to *Agrobacterium* strain LBA4404/pAL4404, the *Agrobacterium* transformants used to inoculate tobacco leaf disks and the resulting transgenic plants regenerated by the methods set out in Example 7.

To assay for expression of the chimeric genes in leaves of the transformed plants, protein was extracted as described in Example 7, with the following modifications. The supernatant from the first ammonium sulfate precipitation, approximately 18 mL, was mixed with an additional 12 mL of cold, saturated ammonium sulfate. The mixture was set on ice for 30 min and centrifuged as described in Example 7. The supernatant was decanted and the pellet, which contained the DHDPS protein, was resuspended in 1 mL of TNE and desalted by passage over a Sephadex G-25 M column (Column PD-10, Pharmacia).

The leaf extracts were assayed for DHDPS activity as described in Example 4. *E. coli* DHDPS could be distinguished from tobacco DHDPS activity by its increased resistance to lysine; E. coli DHDPS retained 80-90% of its activity at 0.1mM lysine, while tobacco DHDPS was completely inhibited at that concentration of lysine. One of ten transformants containing the chimeric gene, 35S promoter/Cab leader/ecodapA/Nos 3', showed *E. coli* DHDPS expression, while five of ten transformants containing the chimeric gene, 35S promoter/Cab leader/cts/ecodapA/Nos 3' showed *E. coli* DHDPS expression.

Free amino acids were extracted from leaves as described in Example 7. Expression of the chimeric gene, 35S promoter/Cab leader/cts/ecodapA/Nos 3', but not 35S promoter/Cab leader/ecodapA/Nos 3' resulted in substantial increases in the level of free lysine in the leaves. Free lysine levels from two to 90-fold higher than untransformed tobacco were observed.

The transformed plants were allowed to flower, self-pollinate and go to seed. Seeds from several lines transformed with the 35S promoter/Cab leader/ cts/ecodapA/Nos 3' gene were surface sterilized and germinated on agar plates in the presence of kanamycin. Lines that showed 3 kanamycin resistant to 1 kanamycin sensitive seedlings, indicative of a single site of insertion of the transgenes, were identified. Progeny that were homozygous for the transgene insert were obtained from these lines using standard genetic analysis. The

homozygous progeny were then characterized for expression of *E. coli* DHDPS in young and mature leaves and for the levels of free amino acids accumulated in young and mature leaves and in mature seeds.

Expression of active *E. coli* DHDPS enzyme was clearly evident in both young and mature leaves of the homozygous progeny of the transformants (Table 4). High levels of free lysine, 50 to 100-fold higher than normal tobacco plants, accumulated in the young leaves of the plants, but a much smaller accumulation of free lysine (2 to 8-fold) was seen in the larger leaves. Experiments that measure lysine in the phloem suggest that lysine is exported from the large leaves. This exported lysine may contribute to the accumulation of lysine in the small growing leaves, which are known to take up, rather than export nutrients. Since the larger leaves make up the major portion of the biomass of the plant, the total increased accumulation of lysine in the plant is more influenced by the level of lysine in the larger leaves. No effect on the free lysine levels in the seeds of these plants was observed (Table 4).

TABLE 4

Progeny of BT463 transformants homozygous for 35S promoter/Cab leader/cts/ecodapA/Nos 3'

LINE	LEAF SIZE	LEAF FREE AMINO ACID K/L	LEAF FREE AMINO ACID K/TOT	E. COLI DHDPS OD/60'/mg	SEED FREE AMINO ACID K/L
NORMAL	3 in.	0.5	0.006	0	0.5
463-18C-2	3 in.	47	0.41	7.6	0.4
463-18C-2	12 in.	1	0.02	5.5	---
463-25A-4	3 in.	58	0.42	6.6	0.4
463-25A-4	12 in.	4	0.02	12.2	---
463-38C-3	3 in.	28	0.28	6.1	0.5
463-38C-3	12 in.	2	0.04	8.3	---

EXAMPLE 10

Transformation of Tobacco with the Phaseolin Promoter/ecodapA Chimeric Genes

The chimeric gene cassettes, phaseolin 5' region/ecodapA/phaseolin 3' region, and phaseolin 5' region/cts/ecodapA/phaseolin 3' region (Example 6) were isolated as approximately 2.6 and 2.8 kb Hind III fragments, respectively. These fragments were inserted into the unique Hind III site of the binary vector pZS97 (Figure 6), yielding pBT506 and pBT534, respectively. This vector is described in Example 8.

The binary vectors containing the chimeric ecodapA genes were transferred by tri-parental matings to *Agrobacterium* strain LBA4404/pAL4404, the *Agrobacterium* transformants used to inoculate tobacco leaf disks and the resulting transgenic plants were regenerated by the methods set out in Example 7.

To assay for expression of the chimeric genes, the transformed plants were allowed to flower, self-pollinate and go to seed. Total seed proteins were extracted as described in Example 8 and immunologically analyzed as described in Example 7, with the following modification. The Western blot membranes were exposed to the DHDPS antibodies prepared in Example 4 at a 1:5000 dilution of the rabbit serum using standard protocol provided by BioRad with their Immun-Blot Kit.

Thirteen of fourteen transformants containing the chimeric gene, phaseolin 5' region/ecodapA/phaseolin 3' region and nine of thirteen transformants containing the chimeric gene, phaseolin 5' region/cts/ecodapA/phaseolin 3' region, produced DHDPS protein detectable via Western blotting (Table 3). Protein which reacted with the DHDPS antibody was of several sizes. Most of the protein was equal in size to DHDPS produced in *E. coli*, whether or not the chimeric gene included the chloroplast transit sequence. This indicated that the chloroplast targeting signal had been efficiently removed from the precursor protein synthesized. This further suggests the majority of the protein entered the chloroplast. In addition, some proteins of lower molecular weight were observed, probably representing breakdown products of the DHDPS polypeptide.

To measure free amino acid composition and total amino acid composition of the seeds, free amino acids and total amino acids were extracted from mature seeds and analyzed as described in Example 8. Expression of either the ecodapA gene or cts/ecodapA had no effect on the total lysine or threonine composition of the seeds in any of the transformed lines (Table 5). Several of the lines that were transformed with the phaseolin 5' region/cts/ecodapA/phaseolin 3' chimeric gene were also tested for any effect on the free amino acid composition. Again, not even a modest effect on the lysine or threonine composition of the seeds was observed in lines expressing high levels of *E. coli* DHDPS protein (Table 5). This was a surprising result, given the dramatic effect (described in Example 9) that expression of this protein has on the free lysine levels in leaves.

One possible explanation for this was that the DHDPS protein observed via Western blot was not functional. To test this hypothesis, total protein extracts were prepared from mature seeds and assayed for DHDPS activity. Approximately 30-40 mg of seeds were put into a 1.5 mL disposable plastic microfuge tube and ground in 0.25 mL of 50 mM Tris-HCl, 50 mM NaCl, 1 mM

EDTA (TNE). The grinding was done using a motorized grinder with disposable plastic shafts designed to fit into the microfuge tube. The resultant suspensions were centrifuged for 5 min at room temperature in a microfuge to remove particulates. Approximately 0.1 mL of aqueous supernatant was removed between the pelleted material and the upper oil phase. The seed extracts were assayed for DHDPS activity as described in Example 4. *E. coli* DHDPS could be distinguished from tobacco DHDPS activity by its increased resistance to lysine; E. coli DHDPS retained about 50% of its activity at 0.4 mM lysine, while tobacco DHDPS was completely inhibited at that concentration of lysine. High levels of *E. coli* DHDPS activity were seen in all four seed extracts tested eliminating this explanation.

The presence of the cts sequence in the chimeric ecodapA gene was essential for eliciting accumulation of high levels of lysine in leaves. Thus another possible explanation was that the cts sequence had somehow been lost during the insertion of the chimeric phaseolin 5' region/cts/ecodapA/phaseolin 3' gene into the binary vector. PCR analysis of several of the transformed lines demonstrated the presence of the cts sequence, however, ruling out this possibility.

A third explanation was that amino acids are not normally synthesized in seeds, and therefore the other enzymes in the pathway were not present in the seeds. The results of experiments presented in Example 8, wherein expression of phaseolin 5' region/cts/lysC-M4/phaseolin 3' gene resulted in accumulation of high levels of free threonine in seeds, indicate that this is not the case.

Taken together these results and the results presented in Example 9, demonstrate that expression of a lysine-insensitive DHDPS in either seeds or leaves is not sufficient to achieve accumulation of increased free lysine in seeds.

TABLE 5

BT506 Transformants: phaseolin 5' region/ecodapA/phaseolin 3'
BT534 Transformants: phaseolin 5' region/cts/ecodapA/phaseolin 3'

	SEED: FREE AMINO ACIDS		SEED: TOTAL AMINO ACIDS		E. COLI DHDPS	
LINE	K/L	T/L	K/L	T/L	0D/60'/MG	WESTERN
NORMAL	0.49	1.34	0.35	0.68		
506-2B			0.34	0.66		+
506-4B			0.33	0.67		+
506-16A			0.34	0.67		+
506-17A			0.36	0.55	7.7	+++
506-19A			0.37	0.45		++

506-22A			0.34	0.67		++
506-23B			0.35	0.67		++
506-33B			0.34	0.67		++
506-38B			0.36	0.69	8.7	+++
506-39A			0.37	0.70		++
506-40A			0.36	0.68		-
506-47A			0.32	0.68		+++
506-48A			0.33	0.69		+++
506-49A			0.33	0.69		+++
534-8A			0.34	0.66		-
534-9A			0.36	0.67		++
534-22B	0.43	1.32	0.39	0.51	4.9	+++
534-31A			0.34	0.66		-
534-38A	0.35	1.49	0.42	0.33		+++
534-39A			0.38	0.69		+
534-7A			0.34	0.67		+++
534-25B			0.35	0.67		+++
534-34B	0.80	1.13	0.42	0.70		-
534-35A	0.43	1.18	0.33	0.67		+++
534-37B	0.42	1.58	0.37	0.68		-
534-43A			0.35	0.68		+++
534-48A	0.46	1.24	0.35	0.68	6.2	+++

EXAMPLE 11

Transformation of Tobacco with the 35S Promoter/cts/dapA plus 35S Promoter/cts/lysC-M4 Chimeric Genes

The 35S promoter/Cab leader/cts/ecodapA/Nos 3', and 35S promoter/Cab leader/cts/lysC-M4/Nos 3' chimeric genes were combined in the binary vector pZS97K (Figure 5). The binary vector is described in Example 7. An oligonucleotide adaptor was synthesized to convert the BamH I site at the 5' end of the 35S promoter/Cab leader/cts/lysC-M4/Nos 3' chimeric gene (see Figure 4a) to an EcoR I site. The 35S promoter/Cab leader/cts/lysC-M4/Nos 3' chimeric gene was then isolated as a 3.6 kb EcoR I fragment from plasmid pBT540 (Example 6) and inserted into pBT463 (Example 9) digested with EcoR I, yielding plasmid pBT564. This vector has both the 35S promoter/Cab leader/cts/ecodapA/Nos 3', and 35S promoter/Cab leader/cts/lysC-M4/Nos 3' chimeric genes inserted in the same orientation.

The binary vector containing the chimeric ecodapA and lysC-M4 genes was transferred by tri-parental matings to *Agrobacterium* strain LBA4404/pAL4404, the *Agrobacterium* transformants used to inoculate tobacco leaf disks and the resulting transgenic plants regenerated by the methods set out in Example 7.

To assay for expression of the chimeric genes in leaves of the transformed plants, protein was extracted as described in Example 7 for AKIII, and as described in Example 9 for DHDPS. The leaf extracts were assayed for DHDPS activity as described in Examples 4 and 9. *E. coli* DHDPS could be distinguished from tobacco DHDPS activity by its increased resistance to lysine; *E. coli* DHDPS retained 80-90% of its activity at 0.1 mM lysine, while tobacco DHDPS was completely inhibited at that concentration of lysine. Extracts were characterized immunologically for expression of AKIII and DHDPS proteins via Western blots as described in Examples 7 and 10.

Ten of twelve transformants expressed *E. coli* DHDPS enzyme activity (Table 6). There was a good correlation between the level of enzyme activity and the amount of DHDPS protein detected immunologically. As described in Example 7, the AK assay was not sensitive enough to detect enzyme activity in these extracts. However, AKIII-M4 protein was detected immunologically in eight of the twelve extracts. In some transformants, 564-21A and 47A, there was a large disparity between the level of expression of DHDPS and AKIII-M4, but in 10 of 12 lines there was a good correlation.

Free amino acids were extracted from leaves and analyzed for amino acid composition as described in Example 7. In the absence of significant AKIII-M4, the level of expression of the chimeric gene, 35S promoter/Cab leader/cts/ecodapA/Nos 3' determined the level of lysine accumulation (Table 6). Compare lines 564-21A, 47A and 39C, none of which expresses significant AKIII-M4. Line 564-21A accumulates about 10-fold higher levels of lysine than line 564-47A which expresses a lower level of *E. coli* DHDPS and 40-fold higher levels of lysine than 564-39C which expresses no *E. coli* DHDPS. However, in transformants that all expressed similar amounts of *E. coli* DHDPS (564-18A, 56A, 36E, 55B, 47A), the level of expression of the chimeric gene, 35S promoter/Cab leader/cts/lysC-M4/Nos 3', controlled the level of lysine accumulation. Thus it is clear that although expression of 35S promoter/Cab leader/cts/lysC-M4/Nos 3' has no effect on the free amino acid levels of leaves when expressed alone (see Example 7), it can increase lysine accumulation when expressed in concert with the 35S promoter/Cab leader/cts/ecodapA/Nos 3' chimeric gene. Expression of these genes together did not effect the level of any other free amino acid in the leaves.

TABLE 6

BT564 Transformants: 35S promoter/Cab leader/cts/ecodapA/Nos 3'
35S promoter/Cab leader/cts/lusC-M4/Nos 3'

LINE	FREE AA LEAF nmol/4mg		FREE AA LEAF		E. COLI DHDPS U/MG/HR	WESTERN DHDPS	WESTERN AK-III
	TOT	K	K/L	K/TOT			
564-21A	117	57	52	0.49	2.4	+++	+/-
564-18A	99	56	69	0.57	1.1	++	++
564-56A	104	58	58	0.56	1.5	++	++
564-36E	85	17	17	0.20	1.5	++	+++
564-55B	54	5	9.1	0.10	1.0	++	+
564-47A	18	1	4.8	0.06	0.8	++	-
564-35A	37	7	13	0.18	0.3	+	++
564-60D	61	3	4.5	0.06	0.2	+	++
564-45A	46	4	8.1	0.09	0.4	+	+
564-44B	50	1	1.7	0.02	0.1	+/-	-
564-49A	53	1	1.0	0.02	0	+/-	-
564-39C	62	1	1.4	0.02	0	-	-

Free amino acids were extracted from mature seeds derived from self-pollinated plants and quantitated as described in Example 8. There was no significant difference in the free amino acid content of seeds from untransformed plants compared to that from the plants showing the highest free lysine accumulation in leaves, i.e. plants 564-18A, 564-21A, 564-36E, 564-56A.

EXAMPLE 12

Transformation of Tobacco with the Phaseolin Promoter/cts/ecodapA plus Phaseolin Promoter/cts/lysC-M4 Chimeric Genes

The chimeric gene cassettes, phaseolin 5' region/cts/ecodapA/phaseolin 3' region and phaseolin 5' region/cts/lysC-M4/phaseolin 3' (Example 6) were combined in the binary vector pZS97 (Figure 6). The binary vector is described in Example 8. To accomplish this the phaseolin 5' region/cts/ecodapA/phaseolin 3' chimeric gene was isolated as a 2.7 kb Hind III fragment and inserted into the Hind III site of vector pUC1318 [Kay et al (1987) *Nucleic Acids Res. 6:*2778], yielding pBT568. It was then possible to digest pBT568 with BamH I and isolate the chimeric gene on a 2.7 kb BamH I fragment. This fragment was inserted into BamH I digested pBT549 (Example 8), yielding pBT570. This binary vector has both chimeric genes, phaseolin 5' region/cts/ecodapA/phaseolin 3' gene and phaseolin 5' region/cts/lysC-M4/phaseolin 3' inserted in the same orientation.

The binary vector pBT570 was transferred by tri-parental mating to *Agrobacterium* strain LBA4404/pAL4404, the *Agrobacterium* transformants used to inoculate tobacco leaf disks and the resulting transgenic plants regenerated by the methods set out in Example 7.

To assay for expression of the chimeric genes in the seeds of the transformed plants, the plants were allowed to flower, self-pollinate and go to seed. Total proteins were extracted from mature seeds and analyzed via western blots as described in Example 8.

Twenty-one of twenty-five transformants expressed the DHDPS protein and nineteen of these also expressed the AKIII protein (Table 7). The amounts of the proteins expressed were related to the number of gene copies present in the transformants; the highest expressing lines, 570-4B, 570-12C, 570-59B and 570-23B, all had two or more sites of insertion of the gene cassette based on segregation of the kanamycin marker gene. Enzymatically active *E. coli* DHDPS was observed in mature seeds of all the lines tested wherein the protein was detected.

To measure free amino acid composition of the seeds, free amino acids were extracted from mature seeds and analyzed as described in Example 8. There was a good correlation between transformants expressing higher levels of both DHDPS and AKIII protein and those having higher levels of free lysine and threonine. The highest expressing lines (marked by asterisk in Table 7) showed up to a 2-fold increase in free lysine levels and up to a 4-fold increase in the level of free threonine in the seeds.

In the highest expressing lines it was possible to detect a high level of α-aminoadipic acid. This compound is known to be an intermediate in the catabolism of lysine in cereal seeds, but is normally detected only via radioactive tracer experiments due to its low level of accumulation. The build-up of high levels of this intermediate indicates that a large amount of lysine is being produced in the seeds of these transformed lines and is passing through the catabolic pathway. The build-up of α-aminoadipic acid was not observed in transformants expressing only *E. coli* DHDPS or only AKIII-M4 in seeds. These results show that it is necessary to express both enzymes simultaneously to produce high levels of free lysine.

TABLE 7

BT570 Transformants: phaseolin 5'region/cts/lysC-M4/phaseolin 3' region
phaseolin 5'region/cts/ecodapA/phaseolin 3' region

LINE	FREE AMINO ACIDS/SEED K/L	T/L	TOTAL AMINO ACIDS/SEED K/L	T/L	WESTERN *E. COLI* DHDPS	WESTERN *E. COLI* AKIII	*E. COLI* DHDPS U/MG/HR	Progeny Kan^r:Kan^s

NORMAL	0.49	1.3	0.35	0.68	-	-		
570-4B	0.31	2.6	0.34	0.64	+++	++		15:1
570-7C	0.39	2.3	0.34	0.64	++	+		
570-8B	0.29	2.1	0.34	0.63	+	-		
570-12C*	0.64	5.1	0.36	0.68	++++	++++	> 4.3	>15:1
570-18A	0.33	3.0	0.35	0.65	++	++		15:1
570-24A	0.33	2.0	0.34	0.65	++	-		
570-37A	0.33	2.1	0.34	0.64	+/-	+/-		
570-44A	0.29	2.1	0.34	0.64	++	+		
570-46B	0.41	2.1	0.35	0.65	++	+		
570-51B	0.33	1.5	0.33	0.64	-	-	0	
570-59B*	0.46	3.0	0.35	0.65	+++	+++	2.6	>15:1
570-80A	0.31	2.2	0.34	0.64	++	+		
570-11A	0.28	2.3	0.34	0.67	++	++		3:1
570-17B	0.27	1.6	0.34	0.65	-	-		
570-20A	0.41	2.3	0.35	0.67	++	+		
570-21B	0.26	2.4	0.34	0.68	++	+		
570-23B*	0.40	3.6	0.34	0.68	+++	+++	3.1	63:1
570-25D	0.30	2.3	0.35	0.66	++	+/-		
570-26A	0.28	1.5	0.34	0.64	-	-		
570-32A	0.25	2.5	0.34	0.67	++	+		
570-35A	0.25	2.5	0.34	0.63	++	++		3:1
570-38A-1	0.25	2.6	0.34	0.64	++	++		3:1
570-38A-3	0.33	1.6	0.35	0.63	-	-		
570-42A	0.27	2.5	0.34	0.62	++	++		3:1
570-45A	0.60	3.4	0.39	0.64	++	++		3:1

* indicates free amino acid sample has α-aminoadipic acid

EXAMPLE 13

Use of the cts/lysC-M4 Chimeric Gene as a Selectable Marker for Tobacco Transformation

The 35S promoter/Cab leader/cts/lysC-M4/Nos 3' chimeric gene in the binary vector pZS97K (pBT542, see Example 7) was used as a selectable genetic marker for transformation of tobacco. High concentrations of lysine plus threonine inhibit growth of shoots from tobacco leaf disks. Expression of active lysine and threonine insensitive AKIII-M4 reverses this growth inhibition (see Example 7).

The binary vector pBT542 was transferred by tri-parental mating to *Agrobacterium* strain LBA4404/pAL4404, the *Agrobacterium* transformants used to inoculate tobacco leaf disks and the resulting transformed shoots were selected on shooting medium containing 3 mM lysine plus 3 mM threonine. Shoots were transferred to rooting media containing 3 mM lysine plus 3 mM threonine. Plants were grown from the rooted shoots. Leaf disks from the plants were placed on shooting medium containing 3 mM lysine plus 3 mM threonine. Transformed plants were identified by the shoot proliferation which occurred around the leaf disks on this medium.

EXAMPLE 14

Transformation of Tobacco with the 35S Promoter/cts/cordapA Chimeric Gene

The 35S promoter/Cab leader/cts/cordapA/Nos 3' chimeric gene was isolated as a 3.0 kb BamH I-Sal I fragment and inserted into BamH I-Sal I digested binary vector pZS97K (Figure 5), yielding plasmid pFS852. The binary vector is described in Example 7.

The binary vector containing the chimeric cordapA gene was transferred by tri-parental mating to *Agrobacterium* strain LBA4404/pAL4404, the *Agrobacterium* transformant was used to inoculate tobacco leaf disks and the resulting transgenic plants were regenerated by the methods set out in Example 7.

To assay for expression of the chimeric gene in leaves of the transformed plants, protein was extracted as described in Example 7, with the following modifications. The supernatant from the first ammonium sulfate precipitation, approximately 18 mL, was mixed with an additional 12 mL of cold, saturated ammonium sulfate. The mixture was set on ice for 30 min and centrifuged as described in Example 7. The supernatant was decanted and the pellet, which contained the DHDPS protein, was resuspended in 1 mL of TNE and desalted by passage over a Sephadex G-25 M column (Column PD-10, Pharmacia).

The leaf extracts were assayed for DHDPS protein and enzyme activity as described in Example 4. *Corynebacteria* DHDPS enzyme activity could be distinguished from tobacco DHDPS activity by its insensitivity to lysine inhibition. Eight of eleven transformants showed *Corynebacteria* DHDPS expression, both as protein detected via western blot and as active enzyme.

Free amino acids were extracted from leaves as described in Example 7. Expression of *Corynebacteria* DHDPS resulted in large increases in the level of free lysine in the leaves (Table 8). However, there was not a good correlation between the level of expression of DHDPS and the amount of free lysine accumulated. Free lysine levels from 2 to 50-fold higher than untransformed

tobacco were observed. There was also a 2 to 2.5-fold increase in the level of total leaf lysine in the lines that showed high levels of free lysine.

TABLE 8

FS586 transformants: 35S promoter/Cab leader/cts/cordapA/Nos 3'

LINE	FREE AMINO ACIDS/LEAF K/L	TOTAL AMINO ACIDS/LEAF K/L	WESTERN CORYNE. DHDPS	CORYNE. DHDPS U/MG/HR
NORMAL	0.5	0.8	-	-
FS586-2A	1.0	0.8	-	-
FS586-4A	0.9	0.8	+	6.1
FS586-11B	3.6	0.8	+	3.4
FS586-11D	26	2.0	+	3.5
FS586-13A	2.4	0.8	+	3.5
FS586-19C	5.1	0.8	+	3.1
FS586-22B	>15	1.5	+	2.3
FS586-30B		0.8	-	-
FS586-38B	18	1.5	++	3.9
FS586-51A	1.3	0.8	-	-
FS586-58C	1.2	0.8	+	5.1

The plants were allowed to flower, self-pollinate and go to seed. Mature seed was harvested and assayed for free amino acid composition as described in Example 8. There was no difference in the free lysine content of the transformants compared to untransformed tobacco seed.

EXAMPLE 15

Transformation of Tobacco with the KTI3 promoter/cts/cordapA or Phaseolin Promoter/cts/cordapA plus Phaseolin Promoter/cts/lysC-M4 Chimeric Genes

The chimeric gene cassettes, KTI3 5' region/cts/ cordapA/KTI3 3' region and phaseolin 5' region/cts/ lysC-M4/phaseolin 3' as well as phaseolin 5' region/cts/ cordapA/phaseolin 3' region and phaseolin 5' region/cts/ lysC-M4/phaseolin 3' (Example 6) were combined in the binary vector pZS97 (Figure 6). The binary vector is described in Example 8.

To accomplish this the KTI3 5' region/cts/cordapA/ KTI3 3' region chimeric gene cassette was isolated as a 3.3 kb BamH I fragment and inserted into BamH I digested pBT549 (Example 8), yielding pFS883. This binary vector has the chimeric genes, KTI3 5' region/cts/cordapA/KTI3 3' region and phaseolin 5' region/cts/lysC-M4/phaseolin 3' region inserted in opposite orientations.

The phaseolin 5' region/cts/cordapA/phaseolin 3'region chimeric gene cassette was modified using oligonucleotide adaptors to convert the Hind III sites at each end to BamH I sites. The gene cassette was then isolated as a 2.7 kb BamH I fragment and inserted into BamH I digested pBT549 (Example 8), yielding pFS903. This binary vector has both chimeric genes, phaseolin 5' region/cts/cordapA/phaseolin 3' region and phaseolin 5' region/cts/lysC-M4/phaseolin 3' region inserted in the same orientation.

The binary vectors pFS883 and pFS903 were transferred by tri-parental mating to *Agrobacterium* strain LBA4404/pAL4404, the *Agrobacterium* transformants were used to inoculate tobacco leaf disks and the resulting transgenic plants were regenerated by the methods set out in Example 7.

To assay for expression of the chimeric genes in the seeds of the transformed plants, the plants were allowed to flower, self-pollinate and go to seed. Total proteins were extracted from mature seeds and analyzed via western blots as described in Example 8.

Twenty-one of twenty-two transformants tested expressed the DHDPS protein and eighteen of these also expressed the AKIII protein (Table 8). Enzymatically active *Corynebacteria* DHDPS was observed in mature seeds of all the lines tested wherein the protein was detected except one.

To measure free amino acid composition of the seeds, free amino acids were extracted from mature seeds and analyzed as described in Example 8. There was a good correlation between transformants expressing higher levels of both DHDPS and AKIII protein and those having higher levels of free lysine and threonine. The highest expressing lines showed up to a 3-fold increase in free lysine levels and up to a 8-fold increase in the level of free threonine in the seeds. As was described in Example 12, a high level of α-aminoadipic acid, indicative of lysine catabolism, was observed in many of the transformed lines (indicated by asterisk in Table 9). There was no major difference in the free amino acid composition or level of protein expression between the transformants which had the KTI3 or Phaseolin regulatory sequences driving expression of the *Corynebacteria* DHDPS gene.

TABLE 9

FS883 Transformants: phaseolin 5' region/cts/lysC-M4/phaseolin 3'
KTI3 5' region/cts/cordapA/KTI3 3'
FS903 Transformants: phaseolin 5' region/cts/lysC-M4/phaseolin 3'
phaseolin 5' region/cts/cordapA/phaseolin 3'

LINE	FREE AMINO ACIDS/SEED K/L	T/L	WESTERN CORYNE. DHDPS	WESTERN *E. COLI* AKIII	CORYNE. DHDPS U/MG/HR	Progeny Kan^r:Kan^s

NORMAL	0.5	1.3	-	-		
FS883-4A	0.9	4.0	+	+		>15:1
FS883-11A	1.0	3.5	++	++	3.1	3:1
FS883-14B	0.5	2.5	++	++		
FS883-16A*	0.7	10.5	+	+++	0	
FS883-17A*	1.0	5.0	+++	+++	7.0	
FS883-18C*	1.2	3.5	++	+	5.8	3:1
FS883-21A	0.5	1.5	+	+/-		
FS883-26B*	1.1	3.6	++	++	2.4	
FS883-29B	0.5	1.5	+	-	0.4	
FS883-32B	0.7	2.4	++	+	1.5	3:1
FS883-38B*	1.1	11.3	+	++	2.0	
FS883-59C*	1.4	6.1	+	+	0.5	15:1
FS903-3C	0.5	1.8	+	+++		
FS903-8A*	0.8	2.1	+++	++++		
FS903-9B	0.6	1.8	++	++	4.3	
FS903-10A	0.5	1.5	-	-		
FS903-22F	0.5	1.8	++	++	0.9	
FS903-35B*	0.8	2.1	++	++		
FS903-36B	0.7	1.5	+	-		
FS903-40A	0.6	1.8	+	+		
FS903-41A*	1.2	2.0	++	+++		
FS903-42A	0.7	2.2	++	+++	5.4	
FS903-44C	0.5	1.9				
FS903-53B	0.6	1.9				

* indicates free amino acid sample has α-aminoadipic acid

Free amino acid composition and expression of bacterial DHDPS and AKIII proteins was also analyzed in developing seeds of two lines that segregated as single gene cassette insertions (see Table 10). Expression of the DHDPS protein under control of the KTI3 promoter was detected at earlier times than that of the AKIII protein under control of the Phaseolin promoter, as expected. At 14 days after flowering both proteins were expressed at a high level and there was about an 8-fold increase in the level of free lysine compared to normal seeds. These results confirm that simultaneous expression of lysine insensitive DHDPS and lysine-insensitive AK results in the production of high levels of free lysine in seeds. Free lysine does not continue to accumulate to even higher levels, however. In mature seeds free lysine is at a level 2 to 3-fold higher than in normal

mature seeds, and the lysine breakdown product α-aminoadipic acid accumulates. These results provide further evidence that lysine catabolism occurs in seeds and prevents accumulation of the high levels of free lysine produced in transformants expressing lysine insensitive DHDPS and lysine insensitive AK.

TABLE 10

Developing seeds of FS883 Transformants:
phaseolin 5' region/cts/lysC-M4/phaseolin 3' region
KTI3 5' region/cts/cordapA/KTI3 3' region

LINE	DAYS AFTER FLOWERING	FREE AMINO ACIDS/SEED K/L	T/L	WESTERN CORYNE. DHDPS	WESTERN *E. COLI* AKIII
FS883-18C	9	1.1	2.1	-	-
FS883-18C	10	1.4	3.3	+/-	-
FS883-18C	11	1.4	2.5	+	-
FS883-18C	14	4.3	1.0	++	++
FS883-18C*	MATURE	1.2	3.5	+++	++
FS883-32B	9	1.3	2.9	+	-
FS883-32B	10	1.6	2.7	+	-
FS883-32B	11	1.4	2.3	+	-
FS883-32B*	14	3.9	1.3	++	++
FS883-32B*	MATURE	0.7	2.4	+++	++

* indicates free amino acid sample has α-aminoadipic acid

EXAMPLE 16

Transformation of Oilseed Rape with the Phaseolin Promoter/cts/cordapA and Phaseolin Promoter/cts/lysC-M4 Chimeric Genes

The chimeric gene cassettes, phaseolin 5' region/ cts/cordapA/phaseolin 3' region, phaseolin 5' region/ cts/lysC-M4/phaseolin 3', and phaseolin 5' region/ cts/cordapA/phaseolin 3' region plus phaseolin 5' region/cts/lysC-M4/phaseolin 3' (Example 6) were inserted into the binary vector pZS199 (Figure 7A), which is similar to pSZ97K described in Example 8. In pZS199 the 35S promoter from Cauliflower Mosaic Virus replaced the Nos promoter driving expression of the NPT II to provide better expression of the marker gene, and the orientation of the polylinker containing the multiple restriction endonuclease sites was reversed.

To insert the phaseolin 5' region/cts/cordapA/ phaseolin 3' region, the gene cassette was isolated as a 2.7 kb BamH I fragment (as described in Example 15) and inserted into BamH I digested pZS199, yielding plasmid pFS926 (Figure 7B). This binary vector has the chimeric gene, phaseolin 5'

region/cts/cordapA/phaseolin 3' region inserted in the same orientation as the 35S/NPT II/nos 3' marker gene.

To insert the phaseolin 5' region/cts/lysC-M4/phaseolin 3' region, the gene cassette was isolated as a 3.3 kb EcoR I to Spe I fragment and inserted into EcoR I plus Xba I digested pZS199, yielding plasmid pBT593 (Figure 7C). This binary vector has the chimeric gene, phaseolin 5' region/cts/lysC-M4/phaseolin 3' region inserted in the same orientation as the 35S/NPT II/nos 3' marker gene.

To combine the two cassettes, the EcoR I site of pBT593 was converted to a BamH I site using oligonucleotide adaptors, the resulting vector was cut with BamH I and the phaseolin 5' region/cts/cordapA/ phaseolin 3' region gene cassette was isolated as a 2.7 kb BamH I fragment and inserted, yielding pBT597 (Figure 7D). This binary vector has both chimeric genes, phaseolin 5' region/cts/cordapA/phaseolin 3' region and phaseolin 5' region/cts/lysC-M4/phaseolin 3' region inserted in the same orientation as the 35S/NPT II/nos 3' marker gene.

Brassica napus cultivar "Westar" was transformed by co-cultivation of seedling pieces with disarmed *Agrobacterium tumefaciens* strain LBA4404 carrying the appropriate binary vector.

B. napus seeds were sterilized by stirring in 10% (v/v) Clorox, 0.1% SDS for thirty min, and then rinsed thoroughly with sterile distilled water. The seeds were germinated on sterile medium containing 30 mM $CaCl_2$ and 1.5% agar, and grown for 6 d in the dark at 24°.

Liquid cultures of *Agrobacterium* for plant transformation were grown overnight at 28°C in Minimal A medium containing 100 mg/L kanamycin. The bacterial cells were pelleted by centrifugation and resuspended at a concentration of 10^8 cells/mL in liquid Murashige and Skoog Minimal Organic medium containing 100 uM acetosyringone.

B. napus seedling hypocotyls were cut into 5 mm segments which were immediately placed into the bacterial suspension. After 30 min, the hypocotyl pieces were removed from the bacterial suspension and placed onto BC-35 callus medium containing 100 uM acetosyringone. The plant tissue and *Agrobacteria* were co-cultivated for 3 d at 24°C in dim light.

The co-cultivation was terminated by transferring the hypocotyl pieces to BC-35 callus medium containing 200 mg/L carbenicillin to kill the *Agrobacteria*, and 25 mg/L kanamycin to select for transformed plant cell growth. The seedling pieces were incubated on this medium for three weeks at 24° under continuous light.

After three weeks, the segments were transferred to BS-48 regeneration medium containing 200 mg/L carbenicillin and 25 mg/L kanamycin. Plant tissue was subcultured every two weeks onto fresh selective regeneration medium, under the same culture conditions described for the callus medium. Putatively transformed calli grew rapidly on regeneration medium; as calli reached a diameter of about 2 mm, they were removed from the hypocotyl pieces and placed on the same medium lacking kanamycin

Shoots began to appear within several weeks after transfer to BS-48 regeneration medium. As soon as the shoots formed discernible stems, they were excised from the calli, transferred to MSV-1A elongation medium, and moved to a 16:8-h photoperiod at 24°.

Once shoots had elongated several internodes, they were cut above the agar surface and the cut ends were dipped in Rootone. Treated shoots were planted directly into wet Metro-Mix 350 soiless potting medium. The pots were covered with plastic bags which were removed when the plants were clearly growing, after about 10 days. Results of the transformation are shown in Table 11. Transformed plants were obtained with each of the binary vectors.

Minimal A Bacterial Growth Medium

Dissolve in distilled water:

10.5	g potassium phosphate, dibasic
4.5	g potassium phosphate, monobasic
1.0	g ammonium sulfate
0.5	g sodium citrate, dihydrate

Make up to 979 mL with distilled water

Autoclave

Add 20 mL filter-sterilized 10% sucrose

Add 1 mL filter-sterilized 1 M MgSO4

Brassica Callus Medium BC-35

Per liter:

Murashige and Skoog Minimal Organic Medium

(MS salts, 100 mg/L i-inositol, 0.4 mg/L thiamine; GIBCO #510-3118)

30 g sucrose

18 g mannitol

0.5 mg/L 2,4-D

0.3 mg/L kinetin

0.6% agarose

pH 5.8

Brassica Regeneration Medium BS-48

Murashige and Skoog Minimal Organic Medium

Gamborg B5 Vitamins (SIGMA #1019)

10 g glucose

250 mg xylose

600 mg MES

0.4% agarose

pH 5.7

Filter-sterilize and add after autoclaving:

2.0 mg/L zeatin

0.1 mg/L IAA

Brassica Shoot Elongation Medium MSV-1A

Murashige and Skoog Minimal Organic Medium

Gamborg B5 Vitamins

10 g sucrose

0.6% agarose

pH 5.8

TABLE 11
Canola transformants

BINARY VECTOR	NUMBER OF CUT ENDS	NUMBER OF KAN^R CALLI	NUMBER OF SHOOTING CALLI	NUMBER OF PLANTS
pZS199	120	41	5	2
pFS926	600	278	52	28
pBT593	600	70	10	3
pBT597	600	223	40	23

Plants were grown under a 16:8-h photoperiod, with a daytime temperature of 23° and a nighttime temperature of 17°. When the primary flowering stem began to elongate, it was covered with a mesh pollen-containment bag to prevent outcrossing. Self-pollination was facilitated by shaking the plants several times each day. Mature seeds derived from self-pollinations were harvested about three months after planting.

A partially defatted seed meal was prepared as follows: 40 mg of mature dry seed was ground with a mortar and pestle under liquid nitrogen to a fine powder. One milliliter of hexane was added and the mixture was shaken at room

temperature for 15 min. The meal was pelleted in an eppendorf centrifuge, the hexane was removed and the hexane extraction was repeated. Then the meal was dried at 65° for 10 min until the hexane was completely evaporated leaving a dry powder. Total proteins were extracted from mature seeds as follows. Approximately 30-40 mg of seeds were put into a 1.5 mL disposable plastic microfuge tube and ground in 0.25 mL of 50 mM Tris-HCl pH 6.8, 2 mM EDTA, 1% SDS, 1% (v/v) β-mercaptoethanol. The grinding was done using a motorized grinder with disposable plastic shafts designed to fit into the microfuge tube. The resultant suspensions were centrifuged for 5 min at room temperature in a microfuge to remove particulates. Three volumes of extract was mixed with 1 volume of 4 X SDS-gel sample buffer (0.1 M Tris-HCl pH6.8, 6.7% SDS, 16.7% (v/v) β-mercaptoethanol, 33% (v/v) glycerol) and 5 μL from each extract were run per lane on an SDS polyacrylamide gel, with bacterially produced DHDPS or AKIII serving as a size standard and protein extracted from untransformed tobacco seeds serving as a negative control. The proteins were then electrophoretically blotted onto a nitrocellulose membrane. The membranes were exposed to the DHDPS or AKIII antibodies at a 1:5000 dilution of the rabbit serum using standard protocol provided by BioRad with their Immun-Blot Kit. Following rinsing to remove unbound primary antibody the membranes were exposed to the secondary antibody, donkey anti-rabbit Ig conjugated to horseradish peroxidase (Amersham) at a 1:3000 dilution. Following rinsing to remove unbound secondary antibody, the membranes were exposed to Amersham chemiluminescence reagent and X-ray film.

Eight of eight FS926 transformants and seven of seven BT597 transformants expressed the DHDPS protein. The single BT593 transformant and five of seven BT597 transformants expressed the AKIII-M4 protein (Table 12). Thus it is straightforward to express these proteins in oilseed rape seeds.

To measure free amino acid composition of the seeds, free amino acids were extracted from 40 mg of the defatted meal in 0.6 mL of methanol/chloroform/water mixed in ratio of 12v/5v/3v (MCW) at room temperature. The mixture was vortexed and then centrifuged in an eppendorf microcentrifuge for about 3 min. Approximately 0.6 mL of supernatant was decanted and an additional 0.2 mL of MCW was added to the pellet which was then vortexed and centrifuged as above. The second supernatant, about 0.2 mL, was added to the first. To this, 0.2 mL of chloroform was added followed by 0.3 mL of water. The mixture was vortexed and then centrifuged in an eppendorf microcentrifuge for about 3 min, the upper aqueous phase, approximately 1.0 mL, was removed, and was dried down in a Savant Speed Vac Concentrator. The

samples were hydrolyzed in 6 N hydrochloric acid, 0.4% (v/v) β-mercaptoethanol under nitrogen for 24 h at 110-120°; 1/4 of the sample was run on a Beckman Model 6300 amino acid analyzer using post-column ninhydrin detection. Relative free amino acid levels in the seeds were compared as ratios of lysine or threonine to leucine, thus using leucine as an internal standard.

In contrast to tobacco seeds, expression of *Corynebacterium* DHDPS lead to large increases in accumulation of free lysine in rapeseed transformants. The highest expressing lines showed a greater than 100-fold increase in free lysine level in the seeds. The transformant that expressed AKIII-M4 in the absence of *Corynebacteria* DHDPS showed a 5-fold increase in the level of free threonine in the seeds. Concomitant expression of both enzymes resulted in accumulation of high levels of free lysine, but not threonine.

A high level of α-aminoadipic acid, indicative of lysine catabolism, was observed in many of the transformed lines. Thus, prevention of lysine catabolism by inactivation of lysine ketoglutarate reductase should further increase the accumulation of free lysine in the seeds. Alternatively, incorporation of lysine into a peptide or lysine-rich protein would prevent catabolism and lead to an increase in the accumulation of lysine in the seeds.

To measure the total amino acid composition of mature seeds, 2 mg of the defatted meal were hydrolyzed in 6 N hydrochloric acid, 0.4% (v/v) β-mercapto-ethanol under nitrogen for 24 h at 110-120°; 1/100 of the sample was run on a Beckman Model 6300 amino acid analyzer using post-column ninhydrin detection. Relative amino acid levels in the seeds were compared as percentages of lysine, threonine or α-aminoadipic acid to total amino acids.

There was a good correlation between expression of DHDPS protein and accumulation of high levels of lysine in the seeds of transformants. Seeds with a 5-100% increase in the lysine level, compared to the untransformed control, were observed. In the transformant with the highest level, lysine makes up about 13% of the total seed amino acids, considerably higher than any previously known rapeseed seed. This transformant expresses high levels of both *E. coli* AKIII-M4 and *Corynebacterium* DHDPS.

TABLE 12

FS926 Transformants: phaseolin 5' region/cts/cordapA/phaseolin 3'
BT593 Transformants: phaseolin 5' region/cts/lysC-M4/phaseolin 3'
BT597 Transformants: phaseolin 5' region/cts/lysC-M4/phaseolin 3'
phaseolin 5' region/cts/cordapA/phaseolin 3'

LINE	FREE AMINO ACIDS K/L	T/L	AA/L	WESTERN CORYNE. DHDPS	WESTERN *E. COLI* AKIII-M4	% TOTAL AMINO ACIDS K	T	AA
WESTAR	0.8	2.0	0	-	-	6.5	5.6	0
ZS199	1.3	3.2	0	-	-	6.3	5.4	0
FS926-3	140	2.0	16	++++	-	12	5.1	1.0
FS926-9	110	1.7	12	++++	-	11	5.0	0.8
FS926-11	7.9	2.0	5.2	++	-	7.7	5.2	0
FS926-6	14	1.8	4.6	+++	-	8.2	5.9	0
FS926-22	3.1	1.3	0.3	+	-	6.9	5.7	0
FS926-27	4.2	1.9	1.1	++	-	7.1	5.6	0
FS926-29	38	1.8	4.7	++++	-	12	5.2	1.6
FS926-68	4.2	1.8	0.9	++	-	8.3	5.5	0
BT593-42	1.4	11	0	-	++	6.3	6.0	0
BT597-14	6.0	2.6	4.3	++	+/-	7.0	5.3	0
BT597-145	1.3	2.9	0	+	-			
BT597-4	38	3.7	4.5	++++	++++	13	5.6	1.6
BT597-68	4.7	2.7	1.5	++	+	6.9	5.8	0
BT597-100	9.1	1.9	1.7	+++	++	6.6	5.7	0
BT597-148	7.6	2.3	0.9	+++	+	7.3	5.7	0
BT597-169	5.6	2.6	1.7	+++	+++	6.6	5.7	0

AA is α-amino adipic acid

EXAMPLE 17

Transformation of Maize Using a Chimeric lysC-M4 Gene as a Selectable Marker

Embryogenic callus cultures were initiated from immature embryos (about 1.0 to 1.5 mm) dissected from kernels of a corn line bred for giving a "type II callus" tissue culture response. The embryos were dissected 10 to 12 d after pollination and were placed with the axis-side down and in contact with agarose-solidified N6 medium [Chu et al. (1974) *Sci Sin 18*:659-668] supplemented with 0.5 mg/L 2,4-D (N6-0.5). The embryos were kept in the dark at 27°C. Friable embryogenic callus consisting of undifferentiated masses of cells with somatic proembryos and somatic embryos borne on suspensor structures proliferated from

the scutellum of the immature embryos. Clonal embryogenic calli isolated from individual embryos were identified and sub-cultured on N6-0.5 medium every 2 to 3 weeks.

The particle bombardment method was used to transfer genes to the callus culture cells. A Biolistic™ PDS-1000/He (BioRAD Laboratories, Hercules, CA) was used for these experiments.

The plasmid pBT573, containing the chimeric gene HH534 5' region/ mcts/lysC-M4/HH2-1 3' region (see Example 6) designed for constitutive gene expression in corn, was precipitated onto the surface of gold particles. To accomplish this 2.5 μg of pBT573 (in water at a concentration of about 1 mg/mL) was added to 25 mL of gold particles (average diameter of 1.5 μm) suspended in water (60 mg of gold per mL). Calcium chloride (25 mL of a 2.5 M solution) and spermidine (10 mL of a 1.0 M solution) were then added to the gold-DNA suspension as the tube was vortexing. The gold particles were centrifuged in a microfuge for 10 s and the supernatant removed. The gold particles were then resuspended in 200 mL of absolute ethanol, were centrifuged again and the supernatant removed. Finally, the gold particles were resuspended in 25 mL of absolute ethanol and sonicated twice for one sec. Five μL of the DNA-coated gold particles were then loaded on each macro carrier disk and the ethanol was allowed to evaporate away leaving the DNA-covered gold particles dried onto the disk.

Embryogenic callus (from the callus line designated #132.2.2) was arranged in a circular area of about 6 cm in diameter in the center of a 100 X 20 mm petri dish containing N6-0.5 medium supplemented with 0.25M sorbitol and 0.25M mannitol. The tissue was placed on this medium for 2 h prior to bombardment as a pretreatment and remained on the medium during the bombardment procedure. At the end of the 2 h pretreatment period, the petri dish containing the tissue was placed in the chamber of the PDS-1000/He. The air in the chamber was then evacuated to a vacuum of 28 inch of Hg. The macrocarrier was accelerated with a helium shock wave using a rupture membrane that bursts when the He pressure in the shock tube reaches 1100 psi. The tissue was placed approximately 8 cm from the stopping screen. Four plates of tissue were bombarded with the DNA-coated gold particles. Immediately following bombardment, the callus tissue was transferred to N6-0.5 medium without supplemental sorbitol or mannitol.

Seven d after bombardment small (2-4 mM diameter) clumps of callus tissue were transferred to N6-0.5 medium lacking casein or proline, but supplemented with 2mM each of lysine and threonine (LT). The tissue continued to grow slowly on this medium and was transferred to fresh N6-0.5 medium

supplemented with LT every 2 weeks. After 12 weeks two clones of actively growing callus was identified on two separate plates containing LT-supplemented medium. These clones continued to grow when sub-cultured on the selective medium. The presence of the lysC-M4 gene in the selected clones was confirmed by PCR analysis. Callus was transferred to medium that promotes plant regeneration.

EXAMPLE 18

Transformation of Corn with the Constitutive Corn Promoter/cts/ecodapA and Constitutive Corn Promoter/cts/lysC-M4

The chimeric gene cassettes, HH534 5' region/ mcts/ecodapA/HH2-1 3' region plus HH534 5' region/ mcts/lysC-M4/HH2-1 3' region, (Example 6) were inserted into the vector pGem9z to generate a corn transformation vector. Plasmid pBT583 (Example 6) was digested with Sal I and an 1850 bp fragment containing the HH534 5' region/mcts/ecodapA/HH2-1 3' region gene cassette was isolated. This DNA fragment was inserted into pBT573 (Example 6), which carries the HH534 5' region/mcts/ lysC-M4/HH2-1 3' region, digested with Xho I. The resulting vector with both chimeric genes in the same orientation was designated pBT586.

Vector pBT586 was introduced into embryogenic corn callus tissue using the particle bombardment method. The establishment of the embryogenic callus cultures and the parameters for particle bombardment were as described in Example 17.

Either one of two plasmid vectors containing selectable markers were used in the transformations. One plasmid, pALSLUC [Fromm et al. (1990) *Biotechnology 8*:833-839], contained a cDNA of the maize acetolactate synthase (ALS) gene. The ALS cDNA had been mutated *in vitro* so that the enzyme coded by the gene would be resistant to chlorsulfuron. This plasmid also contains a gene that uses the 35S promoter from Cauliflower Mosaic Virus and the 3' region of the nopaline synthase gene to express a firefly luciferase coding region [de Wet et al. (1987) *Molec. Cell Biol. 7*:725-737]. The other plasmid, pDETRIC, contained the bar gene from Streptomyces hygroscopicus that confers resistance to the herbicide glufosinate [Thompson et al. (1987 The *EMBO Journal 6*:2519-2523]. The bacterial gene had its translation codon changed from GTG to ATG for proper translation initiation in plants [De Block et al. (1987) The *EMBO Journal 6*:2513-2518]. The bar gene was driven by the 35S promoter from Cauliflower Mosaic Virus and uses the termination and polyadenylation signal from the octopine synthase gene from *Agrobacterium tumefaciens*.

For bombardment, 2.5 μg of each plasmid, pBT586 and one of the two selectable marker plasmids, was co-precipitated onto the surface of gold particles as described in Example 17. Bombardment of the embryogenic tissue cultures was also as described in Example 17.

Seven days after bombardment the tissue was transferred to selective medium. The tissue bombarded with the selectable marker pALSLUC was transferred to N6-0.5 medium that contained chlorsulfuron (30 ng/L) and lacked casein or proline. The tissue bombarded with the selectable marker, pDETRIC, was transferred to N6-0.5 medium that contained 2 mg/L glufosinate and lacked casein or proline. The tissue continued to grow slowly on these selective media. After an additional 2 weeks the tissue was transferred to fresh N6-0.5 medium containing the selective agents.

Chlorsulfuron- and glufosinate-resistance callus clones could be identified after an additional 6-8 weeks. These clones continued to grow when transferred to the selective media.

The presence of pBT586 in the transformed clones has been confirmed by PCR analysis. Functionality of the introduced AK enzyme was tested by plating out transformed clones on N6-0.5 media containing 2 mM each of lysine and threonine (LT selection; see Example 13). All of the clones were capable of growing on LT medium indicating that the *E. coli* aspartate kinase was expressed and was functioning properly. To test that the *E. coli* DHDPS enzyme was functional, transformed callus was plated on N6-0.5 media containing 2μM 2-aminoethylcysteine (AEC), a lysine analog and potent inhibitor of plant DHDPS. The transformed callus tissue was resistant to AEC indicating that the introduced DHDPS, which is about 16-fold less sensitive to AEC than the plant enzyme, was being produced and was functional. Plants have been regenerated from several transformed clones and are being grown to maturity.

EXAMPLE 19

Transformation of Soybean with the Phaseolin Promoter/cts/cordapA and Phaseolin Promoter/cts/lysC-M4 Chimeric Genes

The chimeric gene cassettes, phaseolin 5' region/ cts/cordapA/phaseolin 3' region plus phaseolin 5' region/cts/lysC-M4/phaseolin 3', (Example 6) were inserted into the soybean transformation vector pBT603 (Figure 8A). This vector has a soybean transformation marker gene consisting of the 35S promoter from Cauliflower Mosaic Virus driving expression of the *E. coli* β-glucuronidase gene [Jefferson et al. (1986) *Proc. Natl. Acad. Sci. USA 83*:8447-8451] with the Nos 3' region in a modified pGEM9Z plasmid.

To insert the phaseolin 5' region/cts/lysC-M4/ phaseolin 3' region, the gene cassette was isolated as a 3.3 kb Hind III fragment and inserted into Hind III digested pBT603, yielding plasmid pBT609. This binary vector has the chimeric gene, phaseolin 5' region/ cts/lysC-M4/phaseolin 3' region inserted in the opposite orientation from the 35S/GUS/Nos 3' marker gene.

To insert the phaseolin 5' region/cts/cordapA/ phaseolin 3'region 3', the gene cassette was isolated as a 2.7 kb BamH I fragment (as described in Example 15) and inserted into BamH I digested pBT609, yielding plasmid pBT614 (Figure 8B). This vector has both chimeric genes,
phaseolin 5' region/cts/lysC-M4/phaseolin 3' region and
phaseolin 5' region/cts/cordapA/phaseolin 3' region inserted in the same orientation, and both are in the opposite orientation from the 35S/GUS/Nos 3' marker gene.

Soybean was transformed with plasmid pBT614 according to the procedure described in United States Patent No. 5,015,580. Soybean transformation was performed by Agracetus Company (Middleton, WI). Seeds from five transformed lines were obtained and analyzed.

It was expected that the transgenes would be segregating in the R1 seeds of the transformed plants. To identify seeds that carried the transformation marker gene, a small chip of the seed was cut off with a razor and put into a well in a disposable plastic microtiter plate. A GUS assay mix consisting of 100 mM NaH_2PO_4, 10 mM EDTA, 0.5 mM $K_4Fe(CN)_6$, 0.1% Triton X-100, 0.5 mg/mL 5-Bromo-4-chloro-3-indolyl β-D-glucuronic acid was prepared and 0.15 mL was added to each microtiter well. The microtiter plate was incubated at 37° for 45 min. The development of blue color indicated the expression of GUS in the seed.

Five of seven transformed lines showed approximately 3:1 segregation for GUS expression indicating that the GUS gene was inserted at a single site in the soybean genome. The other transformants showed 9:1 and 15:1 segregation, suggesting that the GUS gene was inserted at two sites.

A meal was prepared from a fragment of individual seeds by grinding into a fine powder. Total proteins were extracted from the meal by adding 1 mg to 0.1 mL of 43 mM Tris-HCl pH 6.8, 1.7% SDS, 4.2% (v/v) β-mercaptoethanol, 8% (v/v) glycerol, vortexing the suspension, boiling for 2-3 min and vortexing again. The resultant suspensions were centrifuged for 5 min at room temperature in a microfuge to remove particulates and 10 μL from each extract were run per lane on an SDS polyacrylamide gel, with bacterially produced DHDPS or AKIII serving as a size standard. The proteins were then electrophoretically blotted onto

a nitrocellulose membrane. The membranes were exposed to the DHDPS or AKIII antibodies, at a 1:5000 or 1:1000 dilution, respectively, of the rabbit serum using standard protocol provided by BioRad with their Immun-Blot Kit. Following rinsing to remove unbound primary antibody the membranes were exposed to the secondary antibody, donkey anti-rabbit Ig conjugated to horseradish peroxidase (Amersham) at a 1:3000 dilution. Following rinsing to remove unbound secondary antibody, the membranes were exposed to Amersham chemiluminescence reagent and X-ray film.

Six of seven transformants expressed the DHDPS protein. In the six transformants that expressed DHDPS, there was excellent correlation between expression of GUS and DHDPS in individual seeds (Table 13). Therefore, the GUS and DHDPS genes are integrated at the same site in the soybean genome. Four of seven transformants expressed the AKIII protein, and again there was excellent correlation between expression of AKIII, GUS and DHDPS in individual seeds (Table 13). Thus, in these four transformants the GUS, AKIII and DHDPS genes are integrated at the same site in the soybean genome. One transformant expressed only GUS in its seeds.

To measure free amino acid composition of the seeds, free amino acids were extracted from 8-10 milligrams of the meal in 1.0 mL of methanol/chloroform/water mixed in ratio of 12v/5v/3v (MCW) at room temperature. The mixture was vortexed and then centrifuged in an eppendorf microcentrifuge for about 3 min; approximately 0.8 mL of supernatant was decanted. To this supernatant, 0.2 mL of chloroform was added followed by 0.3 mL of water. The mixture was vortexed and then centrifuged in an eppendorf microcentrifuge for about 3 min, the upper aqueous phase, approximately 1.0 mL, was removed, and was dried down in a Savant Speed Vac Concentrator. The samples were hydrolyzed in 6 N hydrochloric acid, 0.4% (v/v) β-mercaptoethanol under nitrogen for 24 h at 110-120°; 1/10 of the sample was run on a Beckman Model 6300 amino acid analyzer using post-column ninhydrin detection. Relative free amino acid levels in the seeds were compared as ratios of lysine to leucine, thus using leucine as an internal standard.

Soybean transformants expressing *Corynebacteria* DHDPS alone and in concert with *E. coli* AKIII-M4 accumulated high levels of free lysine in their seeds. From 20 fold to 120-fold increases in free lysine levels were observed (Table 13). A high level of saccharopine, indicative of lysine catabolism, was also observed in seeds that contained high levels of lysine. Thus, prevention of lysine catabolism by inactivation of lysine ketoglutarate reductase should further increase the accumulation of free lysine in the seeds. Alternatively, incorporation

of lysine into a peptide or lysine-rich protein would prevent catabolism and lead to an increase in the accumulation of lysine in the seeds.

To measure the total amino acid composition of mature seeds, 1-1.4 milligrams of the seed meal was hydrolyzed in 6 N hydrochloric acid, 0.4% (v/v) β-mercaptoethanol under nitrogen for 24 h at 110-120°; 1/50 of the sample was run on a Beckman Model 6300 amino acid analyzer using post-column ninhydrin detection. Lysine (and other amino acid) levels in the seeds were compared as percentages of the total amino acids.

The soybean seeds expressing *Corynebacteria* DHDPS showed substantial increases in accumulation of total seed lysine. Seeds with a 5-35% increase in total lysine content, compared to the untransformed control, were observed. In these seeds lysine makes up 7.5-7.7% of the total seed amino acids.

Soybean seeds expressing *Corynebacteria* DHDPS in concert with *E. coli* AKIII-M4 showed much greater accumulation of total seed lysine than those expressing *Corynebacteria* DHDPS alone. Seeds with a more than four-fold increase in total lysine content were observed. In these seeds lysine makes up 20-25% of the total seed amino acids, considerably higher than any previously known soybean seed.

TABLE 13

LINE-SEED	GUS	Free LYS/LEU	DHDPS	AKIII	% TOTAL SEED LYS
A2396-145-4	-	0.9	-	-	5.8
A2396-145-8	-	1.0	-	-	
A2396-145-5	-	0.8			5.9
A2396-145-3	-	1.0			
A2396-145-9	+	2.0			
A2396-145-6	+	4.6			
A2396-145-1	+	8.7			
A2396-145-10	+	18.4			7.5
A2396-145-7	+	21.7	+	-	6.7
A2396-145-2	+	45.5	+	-	7.2
A5403-175-9	-	1.3			
A5403-175-4	-	1.2	-	-	6.0
A5403-175-3	-	1.0	-	-	6.0
A5403-175-7	+	1.5			
A5403-175-5	+	1.8			
A5403-175-1	+	6.2			

A5403-175-2	+	6.5			6.3
A5403-175-6	+	14.4			
A5403-175-8	+	47.8	+	-	7.7
A5403-175-10	+	124.3	+	-	7.5
A5403-181-9	+	1.4			
A5403-181-10	+	1.4	-	-	5.7
A5403-181-8	+	0.9			
A5403-181-6	+	1.5			
A5403-181-4	-	0.7	-	-	5.9
A5403-181-5	+	1.1			
A5403-181-2	-	1.8	-	-	5.6
A5403-181-3	+	2.7	-	-	5.5
A5403-181-7	+	1.9			
A5403-181-1	-	2.3			
A5403-183-9	-	0.8			
A5403-183-6	-	0.7	-	-	6.0
A5403-183-8	-	1.3			
A5403-183-4	-	1.3	-	-	6.0
A5403-183-5	+	0.9			
A5403-183-3	+	3.1			
A5403-183-1	+	3.3			
A5403-183-7	+	9.9			
A5403-183-10	+	22.3	+	+	6.7
A5403-183-2	+	23.1	+	+	7.3
A5403-196-8	-	0.9	-	-	5.9
A5403-196-6	+	8.3			
A5403-196-1	+	16.1	+	+	6.8
A5403-196-7	+	27.9			
A5403-196-3	+	52.8			
A5403-196-5	+	26			
A5403-196-2	+	16.2	+	+	
A5403-196-10	+	29	+	+	7.5
A5403-196-4	+	58.2	+	+	7.6
A5403-196-9	+	47.1			

A2396-233-1	+		+	+	25
A2396-233-2	+				18
A2396-233-3	+				23
A2396-233-4	+				20
A2396-233-5	-		+/-	-	6.0
A2396-233-6	+				16
A2396-233-13	+		+	+	18
A2396-234-1	+		+	+	8.3
A2396-234-2	+		+	+	13
A2396-234-3	+				10
A2396-234-4	+				19
A2396-234-9	+				15
A2396-234-16	-		-	-	5.9
wild type control	-	0.9	-	-	5.6

EXAMPLE 20

Isolation of a Plant Lysine Ketoglutarate Reductase Gene

Lysine Ketoglutarate Reductase (LKR) enzyme activity has been observed in immature endosperm of developing maize seeds [Arruda et al. (1982) *Plant Physiol. 69*:988-989]. LKR activity increases sharply from the onset of endosperm development, reaches a peak level at about 20 d after pollination, and then declines [Arruda et al. (1983) *Phytochemistry 22*:2687-2689].

In order to clone the corn LKR gene, RNA was isolated from developing seeds 19 days after pollination. This RNA was sent to Clontech Laboratories, Inc., (Palo Alto, CA) for the custom synthesis of a cDNA library in the vector Lambda Zap II. The conversion of the Lambda Zap II library into a phagemid library, then into a plasmid library was accomplished following the protocol provided by Clontech. Once converted into a plasmid library the ampicillin-resistant clones obtained carry the cDNA insert in the vector pBluescript SK(-). Expression of the cDNA is under control of the lacZ promoter on the vector.

Two phagemid libraries were generated using the mixtures of the Lambda Zap II phage and the filamentous helper phage of 100 μL to 1 μL. Two additional libraries were generated using mixtures of 100 μL Lambda Zap II to 10 μL helper phage and 20 μL Lambda Zap II to 10 μL helper phage. The titers of the phagemid preparations were similar regardless of the mixture used and were about

2×10^3 ampicillin-resistant-transfectants per mL with *E. coli* strain XL1-Blue as the host and about 1×10^3 with DE126 (see below) as host.

To select clones that carried the LKR gene a specially designed *E. coli* host, DE126 was constructed. Construction of DE126 occurred in several stages. (1) A generalized transducing stock of coliphage P1vir was produced by infection of a culture of TST1 [F^-, araD139, Δ(argF-lac)205, flb5301, ptsF25, relA1, rpsL150, malE52::Tn10, deoC1, λ^-] (E. coli Genetic Stock Center #6137) using a standard method (for Methods see J. Miller, Experiments in Molecular Genetics).

(2) This phage stock was used as a donor in a transductional cross (for Method see J. Miller, Experiments in Molecular Genetics) with strain GIF106M1 [F^-, arg-, ilvA296, lysC1001, thrA1101, metL1000, λ^-, rpsL9, malT1, xyl-7, mtl-2, thi1(?), supE44(?)] (E. coli Genetic Stock Center #5074) as the recipient. Recombinants were selected on rich medium [L supplemented with DAP] containing the antibiotic tetracycline. The transposon Tn10, conferring tetracycline resistance, is inserted in the malE gene of strain TST1. Tetracycline-resistant transductants derived from this cross are likely to contain up to 2 min of the *E. coli* chromosome in the vicinity of malE. The genes malE and lysC are separated by less than 0.5 minutes, well within cotransduction distance.

(3) 200 tetracycline-resistant transductants were thoroughly phenotyped; appropriate fermentation and nutritional traits were scored. The recipient strain GIF106M1 is completely devoid of aspartokinase isozymes due to mutations in thrA, metL and lysC, and therefore requires the presence of threonine, methionine, lysine and meso-diaminopimelic acid (DAP) for growth. Transductants that had inherited $lysC^+$ with malE::Tn10 from TST1 would be expected to grow on a minimal medium that contains vitamin B1, L-arginine, L-isoleucine and L-valine in addition to glucose which serves as a carbon and energy source. Moreover strains having the genetic constitution of $lysC^+$, metL- and thrA- will only express the lysine sensitive aspartokinase. Hence addition of lysine to the minimal medium should prevent the growth of the $lysC^+$ recombinant by leading to starvation for threonine, methionine and DAP. Of the 200 tetracycline resistant transductants examined, 49 grew on the minimal medium devoid of threonine, methionine and DAP. Moreover, all 49 were inhibited by the addition of L-lysine to the minimal medium. One of these transductants was designated DE125. DE125 has the phenotype of tetracycline resistance, growth requirements for arginine, isoleucine and valine, and sensitivity to lysine. The genotype of this strain is F^- malE52::Tn10 arg- ilvA296 thrA1101 metL1000 lambda- rpsL9 malT1 xyl-7 mtl-2 thi1(?) supE44(?).

(4) This step involves production of a male derivative of strain DE125. Strain DE125 was mated with the male strain AB1528 [F'16/delta(gpt-proA)62, lacY1 or lacZ4, glnV44, galK2 rac$^-$(?), hisG4, rfbd1, mgl-51, kdgK51(?), ilvC7, argE3, thi-1] (E. coli Genetic Stock Center #1528) by the method of conjugation. F'16 carries the ilvGMEDAYC gene cluster. The two strains were cross streaked on rich medium permissive for the growth of each strain. After incubation, the plate was replica plated to a synthetic medium containing tetracycline, arginine, vitamin B1 and glucose. DE125 cannot grow on this medium because it cannot synthesize isoleucine. Growth of AB1528 is prevented by the inclusion of the antibiotic tetracycline and the omission of proline and histidine from the synthetic medium. A patch of cells grew on this selective medium. These recombinant cells underwent single colony isolation on the same medium. The phenotype of one clone was determined to be Ilv$^+$, Arg$^-$, TetR, Lysine-sensitive, male specific phage (MS2)-sensitive, consistent with the simple transfer of F'16 from AB1528 to DE125. This clone was designated DE126 and has the genotype F'16/malE52::Tn10, arg$^-$, ilvA296, thrA1101, metL100, lysC$^+$, λ^-, rpsL9, malT1, xyl-7, mtl-2, thi-1?, supE44?. It is inhibited by 20 μg/mL of L-lysine in a synthetic medium.

To select for clones from the corn cDNA library that carried the LKR gene, 100 μL of the phagemid library was mixed with 100 μL of an overnight culture of DE126 grown in L broth and the cells were plated on synthetic media containing vitamin B1, L-arginine, glucose as a carbon and energy source, 100 μg/mL ampicillin and L-lysine at 20, 30 or 40 μg/mL. Four plates at each of the three different lysine concentrations were prepared. The amount of phagemid and DE126 cells was expected to yield about 1 x 10^5 ampicillin-resistant transfectants per plate. Ten to thirty lysine-resistant colonies grew per plate (about 1 lysine-resistant per 5000 ampicillin-resistant colonies).

Plasmid DNA was isolated from 10 independent clones and retransformed into DE126. Seven of the ten DNAs yielded lysine-resistant clones demonstrating that the lysine-resistance trait was carried on the plasmid. Several of the cloned DNAs were sequenced and biochemically characterized. The inserted DNA fragments were found to be derived from the *E. coli* genome, rather than a corn cDNA indicating that the cDNA library provided by Clontech was contaminated.

Another method was used to identify plant cDNAs that encode LKR. This method was based upon expected homology between plant LKR and fungal genes encoding saccharopine dehydrogenase. Fungal saccharopine dehydrogenase (glutamate-forming) and saccharopine dehydrogenase (lysine-forming) catalyze the final two steps in the fungal lysine biosynthetic pathway. Plant LKR and

fungal saccharopine dehydrogenase (lysine-forming) catalyze both forward and reverse reactions, use identical substrates and use similar co-factors. Similarly, plant saccharopine dehydrogenase (glutamate-forming), which catalyzes the second step in the lysine catabolic pathway, works in both forward and reverse reactions, uses identical substrates and uses similar co-factors as fungal saccharopine dehydrogenase (glutamate-forming).

Biochemical and genetic evidence derived from human and bovine studies has demonstrated that mammalian LKR and saccharopine dehydrogenase (glutamate-forming) enzyme activities are present on a single protein with a monomer molecular weight of about 117,000. This contrasts with the fungal enzymes which are carried on separate proteins, saccharopine dehydrogenase (lysine-forming) with a molecular weight of about 44,000 and saccharopine dehydrogenase (glutamate-forming) with a molecular weight of about 51,000. Plant LKR has been reported to have a molecular weight of about 140,000 indicating that it is like the animal catabolic protein wherein both LKR and saccharopine dehydrogenase (glutamate-forming) enzyme activities are present on a single protein.

Several genes for fungal saccharopine dehydrogenases have been isolated and sequenced [Xuan et al. (1990) Mol. Cell. Biol. 10:4795-4806, Feller et al. (1994) *Mol. Cell. Biol. 14*:6411-6418]. The fungal protein sequences, deduced from these gene sequences, were used to search plant cDNA databases for DNA fragments that encoded plant proteins homologous to the fungal saccharopine dehydrogenases. We discovered two plant cDNA fragments from *Arabidopsis thaliana*, SEQ ID NO:102: and SEQ ID NO:103:, that encoded polypeptides SEQ ID NO:104: and SEQ ID NO:105:, respectively, that are homologous to fungal saccharopine dehydrogenase (glutamate-forming). The sequence similarity between the fungal and plant polypeptides (see Figure 9) demonstrate that these cDNAs encode *Arabidopsis* saccharopine dehydrogenase. Oligonucleotides SEQ ID NO:108: and SEQ ID NO:109 were synthesized and used for PCR amplification of a 2.24 kb DNA fragment from genomic *Arabidopsis*. DNA. DNA sequencing of the fragment confirmed that it encoded LKR/SDH. The fragment was labeled with digoxigenin (DIG) using Boehringer Mannheim's Dig-High Prime kit and protocol. This probe was used to screen a CD4-8 Landsberg erecta genomic library by plaque hybridization. Approximately 2.7 X 10^5 recombinant phage were plated on the host *E. coli* LE392, grown overnight at 37°. The protocol was as described in the DIG Wash and Block Set (Boehringer Mannheim) with the hybridization temperature set at 55°. Five positive clones were isolated; one was subcloned into plasmid vector pBluescript ® SK +/-

(Stratagene), transformed into DH5α ™ competent cells (GibcoBRL) and sequenced.

The complete genomic sequence of the *Arabidopsis* LKR/SDH gene is shown in SEQ ID NO:110. The sequence includes approximately 2 kb of 5' noncoding sequence and 500 bp of 3' noncoding sequence and 23 introns. Overlapping fragments of the corresponding cDNA were isolated from total *Arabidopsis* RNA by RT-PCR. Sequence analysis of the LKR-SDH cDNA revealed an ORF of 3.16 kb, which predicts a protein of 117 kd, and confirms that the LKR and SDH enzymes reside on one polypeptide. The complete protein coding sequence of *Arabidopsis* LKR/SDH gene, derived from the cDNA, is shown in SEQ ID NO:111. The deduced amino acid sequence of *Arabidopsis* LKR/SDH protein is shown in SEQ ID NO:112. The protein lacks an N-terminal targeting sequence implying that the lysine degradative pathway is located in the plant cell cytosol.

Degenerate oligonucleotides, SEQ ID NO:113 and SEQ ID NO:114, were designed based upon comparison of the *Arabidopsis* LKR/SDH amino acid sequence with that of other LKR proteins. These were used to amplify soybean and corn LKR/SDH cDNA fragments using PCR from mRNA, or cDNA synthesized from mRNA, isolated from developing soybean or corn seeds. The soybean and corn PCR-generated cDNA fragments were cloned and sequenced. The sequence of the soybean LKR/SDH cDNA fragment is shown in SEQ ID NO:115, and the sequence of the corn cDNA fragment is shown in SEQ ID NO:116. The deduced partial amino acid sequence of soybean LKR/SDH protein is shown in SEQ ID NO:117 and the deduced partial amino acid sequence of corn LKR/SDH protein is shown in SEQ ID NO:118. The partial cDNAs encoding corn and soybean LKR/SDH obtained by PCR, above, were used in protocols that extended the sequence information for these functions. These protocols, which included RACE and direct DNA:DNA hybridization to cDNA libraries for the identification of overlapping clones, are well known to persons skilled in the art. From these efforts, more complete sequences for the corn and soybean cDNAs for LKR/SDH were obtained. SEQ ID NOS:119 and 120 list, respectively, near full-length sequences for the LKR/SDH coding regions from soybean and corn. The deduced protein sequences encoded by these soybean and corn cDNAs are shown in SEQ ID NOS:121 and 122, respectively.

Partial cDNA clones for LKR/SDH from rice and wheat were identifid in libraries prepared from rice roots and leafs and from wheat seedlings. cDNA libraries were prepared in Uni-ZAP™ XR vectors according to the manufacturer's protocol (Stratagene Cloning Systems, La Jolla, CA).

Conversion of the Uni-ZAP™ XR libraries into plasmid libraries was accomplished according to the protocol provided by Stratagene. Upon conversion, cDNA inserts were contained in the plasmid vector pBluescript. cDNA inserts from randomly picked bacterial colonies containing recombinant pBluescript plasmids were amplified via polymerase chain reaction using primers specific for vector sequences flanking the inserted cDNA sequences or plasmid DNA was prepared from cultured bacterial cells. Amplified insert DNAs or plasmid DNAs were sequenced in dye-primer sequencing reactions to generate partial cDNA sequences (expressed sequence tags or "ESTs"; see Adams, M. D. et al., (1991) *Science 252*:1651). The resulting ESTs were analyzed using a Perkin Elmer Model 377 fluorescent sequencer. Possible protein products encoded by the ESTs were compared to the full-length sequence of *Arabidopsis* LKR/SDH (SEQ ID NO:112). A contig for a partial cDNA from rice was constructed and is presented in SEQ ID:125. The predicted prtein fragment from the cDNA contig is shown in SEQ ID NO:126. Another cDNA from rice was identified which corresponds to the 3' end of a LKR/SDH coding region and this sequence is set forth in SEQ ID NO:127. The predicted protein fragment is shown in SEQ ID NO:128. A partial wheat clone was identified and possesses the sequence presented in SEQ ID NO:129. The predicted protein fragment encoded by this cDNA is set forth isn SEQ ID NO:130.

The SDH coding region encompasses 1.4 kb on 3' end of the *Arabidopsis* cDNA clone (SEQ ID NO:131), and encodes a protein of about 52 kD (SEQ ID NO:132). A DNA fragment encoding SDH was generated using PCR primers, which added desired restriction enzyme sites, and ligated into prokaryotic expression vector pBT430 (see Example 2). Addition of the restriction enzyme cleavage site resulted in a change from thr to ala encoded by the second codon. High level expression of *Arabidopsis* SDH was achieved in *E.coli* BL21(DE3)LysS host which expressed T7 RNA polymerase. Extracts from IPTG-induced cells that were transformed with the vector carrying the 1.4 kb insert were analyzed by SDS-PAGE and a protein of the expected size was overproduced in these cells. Separation of the cell extracts into its supernatant (soluble) and pellet (insoluble) fractions showed that substantial amounts of protein were present in both. SDH activity was measured in the soluble fraction of the bacterial extracts. No SDH activity was observed in extracts from cells transformed with an unmodified vector. Extracts from cells containing the SDH cDNA insert converted substantial amounts of NAD+ to NADH. The reaction was specific for SDH because no significant activity was observed in the absence

of the SDH substrate saccharopine. The SDH protein has been purified from these bacterial extracts and used to raise rabbit antibodies to the protein.

In order to block expression of the LKR gene in transformed plants, a chimeric gene designed for cosuppression of LKR is constructed by linking the LKR gene or gene fragment to any of the plant promoter sequences described above. (See U.S. Patent No. 5,231,020 for methodology to block plant gene expression via cosuppression.) The corn LKR gene, SEQ ID NO:120, was modified by introducing an Nco I site at position 7 and a Kpn I site at position 1265 using PCR. This Nco I and Kpn I DNA fragment containing the corn LKR gene fragment was inserted into a plasmid containing the glutelin 2 promoter and 10 kD zein 3' region (see Example 25) to create a chimeric gene for suppression of LKR expression in corn endosperm. The soybean LKR gene, SEQ ID NO:119, was modified by introducing an Nco I site at position 2 and a Kpn I site at position 690 using PCR. This Nco I and Kpn I DNA fragment containing the soybean LKR gene fragment was inserted into a plasmid containing the KTI3 promoter and the KTI3 3' region (see Example 6) to create a chimeric gene for suppression of LKR expression in soybean seeds. Alternatively, a chimeric gene designed to express antisense RNA for all or part of the LKR is constructed by linking the LKR gene or gene fragment in reverse orientation to any of the plant promoter sequences described above. (See U.S. patent 5,107,065 for methodology to block plant gene expression via antisense RNA.) Either the cosuppression or antisense chimeric gene is introduced into plants via transformation as described in other Examples, e.g. Example 18 or Example 19. Transformants wherein expression of the endogenous LKR gene is reduced or eliminated are selected.

EXAMPLE 21

Construction of Synthetic Genes in Expression Vector pSK5

To facilitate the construction and expression of the synthetic genes described below, it was necessary to construct a plasmid vector with the following attributes:

1. No Ear I restriction endonuclease sites such that insertion of sequences would produce a unique site.

2. Containing a tetracycline resistance gene to avoid loss of plasmid during growth and expression of toxic proteins.

3. Containing approximately 290 bp from plasmid pBT430 including the T7 promoter and terminator segment for expression of inserted sequences in *E. coli.*

4. Containing unique EcoR I and Nco I restriction endonuclease recognition sites in proper location behind the T7 promoter to allow insertion of the oligonucleotide sequences.

To obtain attributes 1 and 2 Applicants used plasmid pSK1 which was a spontaneous mutant of pBR322 where the ampicillin gene and the Ear I site near that gene had been deleted. Plasmid pSK1 retained the tetracycline resistance gene, the unique EcoR I restriction sites at base 1 and a single Ear I site at base 2353. To remove the Ear I site at base 2353 of pSK1 a polymerase chain reaction (PCR) was performed using pSK1 as the template. Approximately 10 femtomoles of pSK1 were mixed with 1 μg each of oligonucleotides SM70 and SM71 which had been synthesized on an ABI1306B DNA synthesizer using the manufacturer's procedures.

SM70 5'-CTGACTCGCTGCGCTCGGTC 3' SEQ ID NO:16
SM71 5'-TATTTTCTCCTTACGCATCTGTGC-3' SEQ ID NO:17

The priming sites of these oligonucleotides on the pSK1 template are depicted in Figure 10. The PCR was performed using a Perkin-Elmer Cetus kit (Emeryville, CA) according to the instructions of the vendor on a thermocycler manufactured by the same company. The 25 cycles were 1 min at 95°, 2 min at 42° and 12 min at 72°. The oligonucleotides were designed to prime replication of the entire pSK1 plasmid excluding a 30 b fragment around the Ear I site (see Figure 10). Ten microliters of the 100 μL reaction product were run on a 1% agarose gel and stained with ethidium bromide to reveal a band of about 3.0 kb corresponding to the predicted size of the replicated plasmid.

The remainder of the PCR reaction mix (90 μL) was mixed with 20 μL of 2.5 mM deoxynucleotide triphosphates (dATP, dTTP, dGTP, and dCTP), 30 units of Klenow enzyme added and the mixture incubated at 37° for 30 min followed by 65° for 10 min. The Klenow enzyme was used to fill in ragged ends generated by the PCR. The DNA was ethanol precipitated, washed with 70% ethanol, dried under vacuum and resuspended in water. The DNA was then treated with T4 DNA kinase in the presence of 1 mM ATP in kinase buffer. This mixture was incubated for 30 min at 37° followed by 10 min at 65°. To 10 μL of the kinase-treated preparation, 2 μL of 5X ligation buffer and 10 units of T4 DNA ligase were added. The ligation was carried out at 15° for 16 h. Following ligation, the DNA was divided in half and one half digested with Ear I enzyme. The Klenow,

kinase, ligation and restriction endonuclease reactions were performed as described in Sambrook et al., [*Molecular Cloning, A Laboratory Manual*, 2nd ed. (1989) Cold Spring Harbor Laboratory Press]. Klenow, kinase, ligase and most restriction endonucleases were purchased from BRL. Some restriction endonucleases were purchased from NEN Biolabs (Beverly, MA) or Boehringer Mannheim (Indianapolis, IN). Both the ligated DNA samples were transformed separately into competent JM103 [supE thi Δ(lac-proAB) F' [traD36 proAB, $lacI^q$ lacZ ΔM15] restriction minus] cells using the $CaCl_2$ method as described in Sambrook et al., [*Molecular Cloning, A Laboratory Manual*, 2nd ed. (1989) Cold Spring Harbor Laboratory Press] and plated onto media containing 12.5 μg/mL tetracycline. With or without Ear I digestion the same number of transformants were recovered suggesting that the Ear I site had been removed from these constructs. Clones were screened by preparing DNA by the alkaline lysis miniprep procedure as described in Sambrook et al., [*Molecular Cloning, A Laboratory Manual*, 2nd ed. (1989) Cold Spring Harbor Laboratory Press] followed by restriction endonuclease digest analysis. A single clone was chosen which was tetracycline-resistant and did not contain any Ear I sites. This vector was designated pSK2. The remaining EcoR I site of pSK2 was destroyed by digesting the plasmid with EcoR I to completion, filling in the ends with Klenow and ligating. A clone which did not contain an EcoR I site was designated pSK3.

To obtain attributes 3 and 4 above, the bacteriophage T7 RNA polymerase promoter/terminator segment from plasmid pBT430 (see Example 2) was amplified by PCR. Oligonucleotide primers SM78 (SEQ ID NO:18) and SM79 (SEQ ID NO:19) were designed to prime a 300b fragment from pBT430 spanning the T7 promoter/terminator sequences (see Figure 10).

SM78 5'-TTCATCGATAGGCGACCACACCCGTCC-3' SEQ ID NO:18

SM79 5'-AATATCGATGCCACGATGCGTCCGGCG-3' SEQ ID NO:19

The PCR reaction was carried out as described previously using pBT430 as the template and a 300 bp fragment was generated. The ends of the fragment were filled in using Klenow enzyme and phosphorylated as described above. DNA from plasmid pSK3 was digested to completion with PvuII enzyme and then treated with calf intestinal alkaline phosphatase (Boehringer Mannheim) to remove the 5' phosphate. The procedure was as described in Sambrook et al., [*Molecular Cloning, A Laboratory Manual,* 2nd ed. (1989) Cold Spring Harbor Laboratory Press]. The cut and dephosphorylated pSK3 DNA was purified by

ethanol precipitation and a portion used in a ligation reaction with the PCR generated fragment containing the T7 promoter sequence. The ligation mix was transformed into JM103 [supE thi Δ(lac-proAB) F' [traD36 proAB, $lacI^q$ lacZ ΔM15] restriction minus] and tetracycline-resistant colonies were screened. Plasmid DNA was prepared via the alkaline lysis mini prep method and restriction endonuclease analysis was performed to detect insertion and orientation of the PCR product. Two clones were chosen for sequence analysis: Plasmid pSK5 had the fragment in the orientation shown in Figure 10. Sequence analysis performed on alkaline denatured double-stranded DNA using Sequenase® T7 DNA polymerase (US Biochemical Corp.) and manufacturer's suggested protocol revealed that pSK5 had no PCR replication errors within the T7 promoter/terminator sequence.

The strategy for the construction of repeated synthetic gene sequences based on the Ear I site is depicted in Figure 11. The first step was the insertion of an oligonucleotide sequence encoding a base gene of 14 amino acids. This oligonucleotide insert contained a unique Ear I restriction site for subsequent insertion of oligonucleotides encoding one or more heptad repeats and added an unique Asp 718 restriction site for use in transfer of gene sequences to plant vectors. The overhanging ends of the oligonucleotide set allowed insertion into the unique Nco I and EcoR I sites of vector pSK5.

```
                    M  E  E  K  M  K  A  M  E  E  K
SM81          5'-CATGGAGGAGAAGATGAAGGCGATGGAAGAGAAG
SM80              3'-CTCCTCTTCTACTTCCGCTACCTTCTCTTC
                  NCO I                    EAR I

                  M  K  A                          (SEQ ID NO:22)
SM81              ATGAAGGCGTGATAGGTACCG-3'          (SEQ ID NO:20)
SM80              TACTTCCGCACTATCCATGGCTTAA-5'      (SEQ ID NO:21)
                             ASP718 ECOR I
```

DNA from plasmid pSK5 was digested to completion with Nco I and EcoR I restriction endonucleases and purified by agarose gel electrophoresis. Purified DNA (0.1 μg) was mixed with 1 μg of each oligonucleotide SM80 (SEQ ID NO:14) and SM81 (SEQ ID NO:13) and ligated. The ligation mixture was transformed into *E. coli* strain JM103 [supE thi Δ(lac-proAB) F' [traD36 proAB, $lacI^q$ lacZ ΔM15] restriction minus] and tetracycline resistant transformants screened by rapid plasmid DNA preps followed by restriction digest analysis. A

clone was chosen which had one each of Ear I, Nco I, Asp 718 and EcoR I sites indicating proper insertion of the oligonucleotides. This clone was designated pSK6 (Figure 12). Sequencing of the region of DNA following the T7 promoter confirmed insertion of oligonucleotides of the expected sequence.

Repetitive heptad coding sequences were added to the base gene construct of described above by generating oligonucleotide pairs which could be directly ligated into the unique Ear I site of the base gene. Oligonucleotides SM84 (SEQ ID NO:23) and SM85 (SEQ ID NO:24) code for repeats of the SSP5 heptad. Oligonucleotides SM82 (SEQ ID NO:25) and SM83 (SEQ ID NO:26) code for repeats of the SSP7 heptad.

SSP5	M E E K M K A	(SEQ ID NO:28)
SM84	5'-GATGGAGGAGAAGATGAAGGC-3'	(SEQ ID NO:23)
SM85	3'- CCTCCTCTTCTACTTCCGCTA-5'	(SEQ ID NO:24)
SSP7	M E E K L K A	(SEQ ID NO:27)
SM82	5'-GATGGAGGAGAAGCTGAAGGC-3'	(SEQ ID NO:25)
SM83	3'- CCTCCTCTTCGACTTCCGCTA-5'	(SEQ ID NO:26)

Oligonucleotide sets were ligated and purified to obtain DNA fragments encoding multiple heptad repeats for insertion into the expression vector. Oligonucleotides from each set totaling about 2 μg were phosphorylated, and ligated for 2 h at room temperature. The ligated multimers of the oligonucleotide sets were separated on an 18% non-denaturing 20 X 20 X 0.015 cm polyacrylamide gel (Acrylamide: bis-acrylamide = 19:1). Multimeric forms which separated on the gel as 168 bp (8n) or larger were purified by cutting a small piece of polyacrylamide containing the band into fine pieces, adding 1.0 mL of 0.5 M ammonium acetate, 1 mM EDTA (pH 7.5) and rotating the tube at 37° overnight. The polyacrylamide was spun down by centrifugation, 1 μg of tRNA was added to the supernatant, the DNA fragments were precipitated with 2 volumes of ethanol at -70°, washed with 70% (v/v) ethanol, dried, and resuspended in 10 μL of water.

Ten micrograms of pSK6 DNA were digested to completion with Ear I enzyme and treated with calf intestinal alkaline phosphatase. The cut and dephosphorylated vector DNA was isolated following electrophoresis in a low melting point agarose gel by cutting out the banded DNA, liquefying the agarose at 55°, and purifying over NACS PREPAC columns (BRL) following manufacturer's suggested procedures. Approximately 0.1 μg of purified Ear I

digested and phosphatase treated pSK6 DNA was mixed with 5 μL of the gel purified multimeric oligonucleotide sets and ligated. The ligated mixture was transformed into *E. coli* strain JM103 [supE thi Δ(lac-proAB) F' [traD36 proAB, $lacI^q$ lacZ ΔM15] restriction minus] and tetracycline-resistant colonies selected. Clones were screened by restriction digests of rapid plasmid prep DNA to determine the length of the inserted DNA. Restriction endonuclease analyses were usually carried out by digesting the plasmid DNAs with Asp 718 and Bgl II, followed by separation of fragments on 18% non-denaturing polyacrylamide gels. Visualization of fragments with ethidium bromide, showed that a 150 bp fragment was generated when only the base gene segment was present. Inserts of the oligonucleotide fragments increased this size by multiples of 21 bases. From this screening several clones were chosen for DNA sequence analysis and expression of coded sequences in *E. coli*.

Table 14

Clone #	SEQ ID NO:	Sequence by Heptad Amino Acid Repeat (SSP)	SEQ ID NO:
C15	29	5.7.7.7.7.7.5	30
C20	31	5.7.7.7.7.7.5	32
C30	33	5.7.7.7.7.5	34
D16	35	5.5.5.5	36
D20	37	5.5.5.5.5	38
D33	39	5.5.5.5	40

The first and last SSP5 heptads flanking the sequence of each construct are from the base gene described above. Inserts are designated by underlining.

Because the gel purification of the oligomeric forms of the oligonucleotides did not give the expected enrichment of longer (i.e., >8n) inserts, Applicants used a different procedure for a subsequent round of insertion constructions. For this series of constructs four more sets of oligonucleotides were generated which code for SSP 8,9,10 and 11 amino acid sequences respectively:

```
SSP8          M  E  E  K  L  K  K                (SEQ ID NO:49)
SM86     5'-GATGGAGGAGAAGCTGAAGAA-3'              (SEQ ID NO:41)
SM87     3'-   CCTCCTCTTCGACTTCTTCTA-5'           (SEQ ID NO:42)
```

SSP9	M E E K L K W	(SEQ ID NO:50)
SM88	5'-GATGGAGGAGAAGCTGAAGTG-3'	(SEQ ID NO:43)
SM89	3'- CCTCCTCTTCGACTTCACCTA-5'	(SEQ ID NO:44)
SSP10	M E E K M K K	(SEQ ID NO:51)
SM90	5'-GATGGAGGAGAAGATGAAGAA-3'	(SEQ ID NO:45)
SM91	3'- CCTCCTCTTCTACTTCTTCTA-5'	(SEQ ID NO:46)
SSP11	M E E K M K W	(SEQ ID NO:52)
SM92	5'-GATGGAGGAGAAGATGAAGTG-3'	(SEQ ID NO:47)
SM93	3'- CCTCCTCTTCTACTTCACCTA-5'	(SEQ ID NO:48)

The following HPLC procedure was used to purify multimeric forms of the oligonucleotide sets after phosphorylating and ligating the oligonucleotides as described above. Chromatography was performed on a Hewlett Packard Liquid Chromatograph instrument, Model 1090M. Effluent absorbance was monitored at 260 nm. Ligated oligonucleotides were centrifuged at 12,000xg for 5 min and injected onto a 2.5 micron TSK DEAE-NPR ion exchange column (35 cm x 4.6 mm i.d.) fitted with a 0.5 micron in-line filter (Supelco). The oligonucleotides were separated on the basis of length using a gradient elution and a two buffer mobile phase [Buffer A: 25 mM Tris-Cl, pH 9.0, and Buffer B: Buffer A + 1 M NaCl]. Both Buffers A and B were passed through 0.2 micron filters before use. The following gradient program was used with a flow rate of 1 mL per min at 30°:

Time	%A	%B
initial	75	25
0.5 min	55	45
5 min	50	50
20 min	38	62
23 min	0	100
30 min	0	100
31 min	75	25

Fractions (500 µL) were collected between 3 min and 9 min. Fractions corresponding to lengths between 120 bp and 2000 bp were pooled as determined from control separations of restriction digests of plasmid DNAs.

The 4.5 mL of pooled fractions for each oligonucleotide set were precipitated by adding 10 µg of tRNA and 9.0 mL of ethanol, rinsed twice with 70% ethanol and resuspended in 50 µL of water. Ten µL of the resuspended

HPLC purified oligonucleotides were added to 0.1 μg of the Ear I cut, dephosphorylated pSK6 DNA described above and ligated overnight at 15°. All six oligonucleotide sets described above which had been phosphorylated and self-ligated but not purified by gel or HPLC were also used in separate ligation reactions with the pSK6 vector. The ligation mixtures were transformed into *E. coli* strain DH5α [supE44 ΔlacU169 (Φ80 lacZ ΔM15) hsdR17 recA1 endA1 gyr196 thi1 relA1] and tetracycline-resistant colonies selected. Applicants chose to use the DH5α [supE44 ΔlacU169 (Φ80 lacZ ΔM15) hsdR17 recA1 endA1 gyr196 thi1 relA1] strain for all subsequent work because this strain has a very high transformation rate and is recA-. The recA- phenotype eliminates concerns that these repetitive DNA structures may be substrates for homologous recombination leading to deletion of multimeric sequences.

Clones were screened as described above. Several clones were chosen to represent insertions of each of the six oligonucleotide sets.

Table 15

Clone #	SEQ ID NO:	Sequence by Heptad Amino Acid Repeat (SSP)	SEQ ID NO:
82-4	53	7.7.7.7.7.7.5	54
84-H3	55	5.5.5.5	56
86-H23	57	5.8.8.5	58
88-2	59	5.9.9.9.5	60
90-H8	61	5.10.10.10.5	62
92-2	63	5.11.11.5	64

The first and last SSP5 heptads flanking the sequence represent the base gene sequence. Insert sequences are underlined. Clone numbers including the letter "H" designate HPLC-purified oligonucleotides. The loss of the first base gene repeat in clone 82-4 may have resulted from homologous recombination between the base gene repeats 5.5 before the vector pSK6 was transferred to the recA- strain. The HPLC procedure did not enhance insertion of longer multimeric forms of the oligonucleotide sets into the base gene but did serve as an efficient purification of the ligated oligonucleotides.

Oligonucleotides were designed which coded for mixtures of the SSP sequences and which varied codon usage as much as possible. This was done to reduce the possibility of deletion of repetitive inserts by recombination once the synthetic genes were transformed into plants and to extend the length of the constructed gene segments. These oligonucleotides encode four repeats of heptad coding units (28 amino acid residues) and can be inserted at the unique Ear I site

in any of the previously constructed clones. SM96 and SM97 code for $SSP(5)_4$, SM98 and SM99 code for $SSP(7)_4$ and SM100 plus SM101 code for SSP8.9.8.9.

```
                    M  E  E  K  M  K  A  M  E  E  K  M  K
      SM96        5'-GATGGAGGAAAAGATGAAGGCGATGGAGGAGAAAATGAAA
      SM97        3'    CCTCCTTTTCTACTTCCGCTACCTCCTCTTTTACTTT
A  M  E  E  K  M  K  A  M  E  E  K  M  K  A          (SEQ ID NO:67)
GCTATGGAGGAAAAGATGAAAGCGATGGAGGAGAAAATGAAGGC-3'     (SEQ ID NO:65)
CGATACCTCCTTTTCTACTTTCGCTACCTCCTCTTTTACTTCCGCTA-5'  (SEQ ID NO:66)
```

```
                    M  E  E  K  L  K  A  M  E  E  K  L  K
      SM98        5'-GATGGAGGAAAAGCTGAAAGCGATGGAGGAGAAACTCAAG
      SM99        3'    CCTCCTTTTCGACTTTCGCTACCTCCTCTTTGAGTTC
   A  M  E  E  K  L  K  A  M  E  E  K  L  K  A       (SEQ ID NO:70)
   GCTATGGAAGAAAAGCTTAAAGCGATGGAGGAGAAACTGAAGGC-3'   (SEQ ID NO:68)
   CGATACCTTCTTTTCGAATTTCGCATCCTCCTCTTTGACTTCCGCTA-5' (SEQ ID NO:69)
```

```
                    M  E  E  K  L  K  K  M  E  E  K  L  K
      SM100       5'-GATGGAGGAAAAGCTTAAGAAGATGGAAGAAAAGCTGAAA
      SM101       3'    CCTCCTTTTCGAATTCTTCTACCTTCTTTTCGACTTT
   W  M  E  E  K  L  K  K  M  E  E  K  L  K  W       (SEQ ID NO:73)
   TGGATGGAGGAGAAACTCAAAAAGATGGAGGAAAAGCTTAAATG-3'   (SEQ ID NO:71)
   ACCTACCTCCTCTTTGAGTTTTTCATCCTCCTTTTCGAATTTACCTA-5' (SEQ ID NO:72)
```

DNA from clones 82-4 and 84-H3 were digested to completion with Ear I enzyme, treated with phosphatase and gel purified. About 0.2 μg of this DNA were mixed with 1.0 μg of each of the oligonucleotide sets SM96 and SM97, SM98 and SM99 or SM100 and SM101 which had been previously phosphorylated. The DNA and oligonucleotides were ligated overnight and then the ligation mixes transformed into *E. coli* strain DH5α. Tetracycline-resistant colonies were screened as described above for the presence of the oligonucleotide inserts. Clones were chosen for sequence analysis based on their restriction endonuclease digestion patterns.

Table 16

Clone #	SEQ ID NO:	Sequence by Heptad Amino Acid Repeat (SSP)	SEQ ID NO:
2-9	74	7.7.7.7.7.7.8.9.8.9.5	75
3-5	78	7.7.7.7.7.7.5.5	79
5-1	76	5.5.5.7.7.7.7.5	77

Inserted oligonucleotide segments are underlined

Clone 2-9 was derived from oligonucleotides SM100 (SEQ ID NO:71) and SM101 (SEQ ID NO:72) ligated into the Ear I site of clone 82-4 (see above). Clone 3-5 (SEQ ID NO:78) was derived from the insertion of the first 22 bases of the oligonucleotide set SM96 (SEQ ID NO:65) and SM97 (SEQ ID NO:66) into the Ear I site of clone 82-4 (SEQ ID NO:53). This partial insertion may reflect improper annealing of these highly repetitive oligos. Clone 5-1 (SEQ ID NO:76) was derived from oligonucleotides SM98 (SEQ ID NO:68) and SM99 (SEQ ID NO:69) ligated into the Ear I site of clone 84-H3 (SEQ ID NO:55).

Strategy II.

A second strategy for construction of synthetic gene sequences was implemented to allow more flexibility in both DNA and amino acid sequence. This strategy is depicted in Figure 13 and Figure 14. The first step was the insertion of an oligonucleotide sequence encoding a base gene of 16 amino acids into the original vector pSK5. This oligonucleotide insert contained an unique Ear I site as in the previous base gene construct for use in subsequent insertion of oligonucleotides encoding one or more heptad repeats. The base gene also included a BspH I site at the 3' terminus. The overhanging ends of this cleavage site are designed to allow "in frame" protein fusions using Nco I overhanging ends. Therefore, gene segments can be multiplied using the duplication scheme described in Figure 14. The overhanging ends of the oligonucleotide set allowed insertion into the unique Nco I and EcoR I sites of vector pSK5.

```
                  M  E  E  K  M  K  K  L  E  E  K
SM107       5'-CATGGAGGAGAAGATGAAAAAGCTCGAAGAGAAG
SM106            3'-CTCCTCTTCTACTTTTTCGAGCTTCTCTTC
                 NCO I                      EAR I
```

```
                M  K  V  M  K                              (SEQ ID NO:82)
                ATGAAGGTCATGAAGTGATAGGTACCG-3'          (SEQ ID NO:80)
                TACTTCCAGTACTTCACTATCCATGGCTTAA-5'      (SEQ ID NO:81)
                      BSPH I  ASP 718
```

The oligonucleotide set was inserted into pSK5 vector as described in Strategy I above. The resultant plasmid was designated pSK34.

Oligonucleotide sets encoding 35 amino acid "segments" were ligated into the unique Ear I site of the pSK34 base gene using procedures as described above. In this case, the oligonucleotides were not gel or HPLC purified but simply annealed and used in the ligation reactions. The following oligonucleotide sets were used:

```
SEG 3           L  E  E  K  M  K  A  M  E  D  K  M  K  W
SM110       5'-GCTGGAAGAAAAGATGAAGGCTATGGAGGACAAGATGAAATGG
SM111          3'-CCTTCTTTTCTACTTCCGATACCTCCTGTTCTACTTTACC

                L  E  E  K  M  K  K                    (SEQ ID NO:85)
                                                  (amino acids 8-28)
                CTTGAGGAAAAGATGAAGAA-3'                (SEQ ID NO:83)
                GAACTCCTTTTCTACTTCTTCGA-5'              (SEQ ID NO:84)

SEG 4           L  E  E  K  M  K  A  M  E  D  K  M  K  W
SM112       5'-GCTCGAAGAAAGATGAAGGCAATGGAAGACAAAATGAAGTGG
SM113          3'-GCTTCTTTCTACTTCCGTTACCTTCTGTTTTACTTCACC

                L  E  E  K  M  K  K                    (SEQ ID NO:86)
                                                  (amino acids 8-28)
                CTTGAGGAGAAAATGAAGAA-3'                (SEQ ID NO:87)
                GAACTCCTCTTTTACTTCTTCGA-5'              (SEQ ID NO:88)

SEG 5           L  K  E  E  M  A  K  M  K  D  E  M  W  K
SM114       5'-GCTCAAGGAGGAAATGGCTAAGATGAAAGACGAAATGTGGAAA
SM115          3'-GTTCCTCCTTTACCGATTCTACTTTCTGCTTTACACCTTT

                L  K  E  E  M  K  K                    (SEQ ID NO:89)
                                                  (amino acids 8-28)
                CTGAAAGAGGAAATGAAGAA                   (SEQ ID NO:90)
                GACTTTCTCCTTTACTTCTTCGA                 (SEQ ID NO:91)
```

Clones were screened for the presence of the inserted segments by restriction digestion followed by separation of fragments on 6% acrylamide gels. Correct insertion of oligonucleotides was confirmed by DNA sequence analyses. Clones containing segments 3, 4 and 5 respectively were designated pSKseg3, pSKseg4, and pSKseg5.

These "segment" clones were used in a duplication scheme as shown in Figure 14. Ten μg of plasmid pSKseg3 were digested to completion with Nhe I and BspH I and the 1503 bp fragment isolated from an agarose gel using the Whatman paper technique. Ten μg of plasmid pSKseg4 were digested to completion with Nhe I and Nco I and the 2109 bp band gel isolated. Equal amounts of these fragments were ligated and recombinants selected on tetracycline. Clones were screened by restriction digestions and their sequences confirmed. The resultant plasmid was designated pSKseg34.

pSKseg34 and pSKseg5 plasmid DNAs were digested, fragments isolated and ligated in a similar manner as above to create a plasmid containing DNA sequences encoding segment 5 fused to segments 3 and 4. This construct was designated pSKseg534 and encodes the following amino acid sequence:

SSP534 NH2-MEEKMKKLKEEMAKMKDEMWKLKEEMKKLEEKMKVMEEKMKKLEEKMKA
MEDKMKWLEEKMKKLEEKMKVMEEKMKKLEEKMKAMEDKMKWLEEKMKK
LEEKMKVMK-COOH (SEQ ID NO:92)

EXAMPLE 22

Construction of SSP Chimeric Genes for Expression in the Seeds of Plants

To express the synthetic gene products described in Example 21 in plant seeds, the sequences were transferred to the seed promoter vectors pCW108, pCW109 or pML113 (Figure 15). The vectors pCW108 and pML113 contain the bean phaseolin promoter (from base +1 to base -494),and 1191 bases of the 3' sequences from bean phaseolin gene. Plasmid pCW109 contains the soybean β-conglycinin promoter (from base +1 to base -619) and the same 1191 bases of 3' sequences from the bean phaseolin gene. These vectors were designed to allow direct cloning of coding sequences into unique Nco I and Asp 718 sites. These vectors also provide sites (Hind III or Sal I) at the 5' and 3' ends to allow transfer of the promoter/coding region/3' sequences directly to appropriate binary vectors.

To insert the synthetic storage protein gene sequences, 10 μg of vector DNA were digested to completion with Asp 718 and Nco I restriction endonucleases. The linearized vector was purified via electrophoresis on a 1.0% agarose gel overnight electrophoresis at 15 volts. The fragment was collected by cutting the agarose in front of the band, inserting a 10 X 5 mm piece of Whatman 3MM paper into the agarose and electrophoresing the fragment into the paper [Errington,

(1990) Nucleic Acids Research, 18:17]. The fragment and buffer were spun out of the paper by centrifugation and the DNA in the ~100 μL was precipitated by adding 10 mg of tRNA, 10 μL of 3 M sodium acetate and 200 μL of ethanol. The precipitated DNA was washed twice with 70% ethanol and dried under vacuum. The fragment DNA was resuspended in 20 μL of water and a portion diluted 10-fold for use in ligation reactions.

Plasmid DNA (10 mg) from clone 3-5 (carrying the SSP3-5 coding sequence) and pSK534 (carrying the SSP534 coding sequence) was digested to completion with Asp 718 and Nco I restriction endonucleases. The digestion products were separated on an 18% polyacrylamide non-denaturing gel. Gel slices containing the desired fragments were cut from the gel and purified by inserting the gel slices into a 1% agarose gel and electrophoresing for 20 min at 100 volts. DNA fragments were collected on 10 X 5 mm pieces of Whatman 3MM paper, the buffer and fragments spun out by centrifugation and the DNA precipitated with ethanol. The fragments were resuspended in 6 μL water. One microliter of the diluted vector fragment described above, 2 μL of 5X ligation buffer and 1 μL of T4 DNA ligase were added. The mixture was ligated overnight at 15°.

The ligation mixes were transformed into *E. coli* strain DH5α [supE44 ΔlacU169 (Φ80 lacZ ΔM15) hsdR17 recA1 endA1 gyr196 thi1 relA1] and ampicillin-resistant colonies selected. The clones were screened by restriction endonuclease digestion analyses of rapid plasmid DNAs and by DNA sequencing.

EXAMPLE 23

Tobacco Plants Containing the Chimeric Genes Phaseolin Promoter/cts/lysC-M4 and β-conglycinin promoter/SSP3-5

The binary vector pZS97 was used to transfer the chimeric SSP3-5 gene of Example 22 and the chimeric *E. coli* dapA and lysC-M4 genes of Example 4 to tobacco plants. Binary vector pZS97 (Figure 6) is part of a binary Ti plasmid vector system [Bevan, (1984) *Nucl. Acids. Res. 12*:8711-8720] of *Agrobacterium tumefaciens*. The vector contains: (1) the chimeric gene nopaline synthase::neomycin phosphotransferase (nos::NPTII) as a selectable marker for transformed plant cells [Bevan et al., (1983) Nature 304:184-186], (2) the left and right borders of the T-DNA of the Ti plasmid [Bevan, (1984) Nucl. Acids. Res. 12:8711-8720], (3) the *E. coli* lacZ α-complementing segment [Viera et al., (1982) *Gene 19*:259-267] with a unique Sal I site(pSK97K) or unique Hind III site (pZS97) in the polylinker region, (4) the bacterial replication origin from the *Pseudomonas* plasmid pVS1 [Itoh et al., (1984) *Plasmid 11*:206-220], and (5) the bacterial β–lactamase gene as a selectable marker for transformed *A. tumefaciens*.

Plasmid pZS97 DNA was digested to completion with Hind III enzyme and the digested plasmid was gel purified. The Hind III digested pZS97 DNA was mixed with the Hind III digested and gel isolated chimeric SSP3-5 gene of Example 22, ligated, transformed and colonies selected on ampicillin.

The binary vector containing the chimeric gene was transferred by tri-parental mating [Ruvkin et al., (1981) *Nature 289*:85-88] to *Agrobacterium* strain LBA4404/pAL4404 [Hockema et al., (1983), *Nature 303*:179-180] selecting for carbenicillin resistance. Cultures of *Agrobacterium* containing the binary vector was used to transform tobacco leaf disks [Horsch et al., (1985) *Science 227*:1229-1231]. Transgenic plants were regenerated in selective medium containing kanamycin.

Transformed tobacco plants containing the chimeric gene, β-conglycinin promoter/SSP3-5/phaseolin 3' region, were thus obtained. Two transformed lines, pSK44-3A and pSK44-9A, which carried a single site insertion of the SSP3-5 gene were identified based upon 3:1 segregation of the marker gene for kanamycin resistance. Progeny of the primary transformants, which were homozygous for the transgene, pSK44-3A-6 and pSK44-9A-5, were then identified based upon 4:0 segregation of the kanamycin resistance in seeds of these plants.

Similarly, transformed tobacco plants with the chimeric genes phaseolin 5' region/cts/lysC-M4/phaseolin 3' region and phaseolin 5' region/cts/ecodapA/phaseolin 3' region were obtained as described in Example 12. A transformed line, BT570-45A, which carried a single site insertion of the DHDPS and AK genes was identified based upon 3:1 segregation of the marker gene for kanamycin resistance. Progeny from the primary transformant which were homozygous for the transgene, BT570-45A-3 and BT570-45A-4, were then identified based upon 4:0 segregation of the kanamycin resistance in seeds of these plants.

To generate plants carrying all three chimeric genes genetic crosses were performed using the homozygous parents. Plants were grown to maturity in greenhouse conditions. Flowers to be used as male and female were selected one day before opening and older flowers on the inflorescence removed. For crossing, female flowers were chosen at the point just before opening when the anthers were not dehiscent. The corolla was opened on one side and the anthers removed. Male flowers were chosen as flowers which had opened on the same day and had dehiscent anthers shedding mature pollen. The anthers were removed and used to pollinate the pistils of the anther-stripped female flowers. The pistils were then covered with plastic tubing to prevent further pollination. The seed pods were

allowed to develop and dry for 4-6 weeks and harvested. Two to three separate pods were recovered from each cross. The following crosses were performed:

Male	X	Female
BT570-45A-3		pSK44-3A-6
BT570-45A-4		pSK44-3A-6
pSK44-3A-6		BT570-45A-4
BT570-45A-5		pSK44-9A-5
pSk44-9A-5		BT570-45A-5

Dried seed pods were broken open and seeds collected and pooled from each cross. Thirty seeds were counted out for each cross and for controls seeds from selfed flowers of each parent were used. Duplicate seed samples were hydrolyzed and assayed for total amino acid content as described in Example 8. The amount of increase in lysine as a percent of total seeds amino acids over wild type seeds, which contain 2.56% lysine, is presented in Table 16 along the copy number of each gene in the endosperm of the seed.

TABLE 17

male	X	female	copy number AK & DHDPS genes	copy number SSP gene	lysine increase
BT570-45A	X	BT570-45A	1*	0	0
pSK44-9A	X	pSK44-9A	0	1*	0.12
pSK44-9A-5	X	pSK44-9A-5	0	2	0.29
pSK44-9A-5	X	BT570-45A-5	1	1	0.6
BT570-45A-5	X	pSK44-9A-5	1	1	0.29
pSK44-3A	X	pSK44-3A	0	1*	0.28
pSK44-3A-6	X	pSK44-3A-6	0	2	0.5
pSK44-3A-6	X	BT570-45A-4	1	1	0.62
BT570-45A-3	X	pSK44-3A-6	1	1	0.27
BT570-45A-4	X	pSK44-3A-6	1	1	0.29

* copy number is average in population of seeds

The results of these crosses demonstrate that the total lysine levels in seeds can be increased by the coordinate expression of the lysine biosynthesis genes and the high lysine protein SSP3-5. In seeds derived from hybrid tobacco plants, this synergism is strongest when the biosynthesis genes are derived from the female parent. It is expected that the lysine level would be further increased if the biosynthesis genes and the lysine-rich protein genes were all homozygous.

EXAMPLE 24

Soybean Plants Containing the Chimeric Genes Phaseolin Promoter/cts/cordapA, Phaseolin Promoter/cts/lysC-M4 and Phaseolin Promoter/SSP3-5

Transformed soybean plants that express the chimeric gene, phaseolin promoter/cts/cordapA/ phaseolin 3' region and phaseolin promoter/cts/lysC-M4/ phaseolin 3' region have been described in Example 19. Transformed soybean plants that express the chimeric gene, phaseolin promoter/SSP3-5/phaseolin 3' region, were obtained by inserting the chimeric gene as an isolated Hind III fragment into an equivalent soybean transformation vector plasmid pML63 (Figure 16) and carrying out transformation as described in Example 19.

Seeds from primary transformants were sampled by cutting small chips from the sides of the seeds away from the embryonic axis. The chips were assayed for GUS activity as described in Example 19 to determine which of the segregating seeds carried the transgenes. Half seeds were ground to meal and assayed for expression of SSP3-5 protein by Enzyme Linked ImmunoSorbent Assay (ELISA). was performed as follows:

A fusion protein of glutathione-S-transferase and the SSP3-5 gene product was generated through the use of the Pharmacia_ pGEX GST Gene Fusion System (*Current Protocols in Molecular Biology, Vol. 2*, pp 16.7.1-8, (1989) John Wiley and Sons). The fusion protein was purified by affinity chromatography on glutathione agarose (Sigma) or glutathione Sepharose (Pharmacia) beads, concentrated using Centricon 10 (Amicon) filters, and then subjected to SDS polyacrylamide electrophoresis (15% acrylamide, 19:1 acrylamide:bisacrylamide) for further purification. The gel was stained with Coomassie Blue for 30 min, destained in 50% (v/v) methanol, 10% (v/v) acetic acid and the protein bands electroeluted using an Amicon Centriluter Microelectroeluter (Paul T. Matsudaira ed., *A Practical Guide to Protein and Peptide Purification for Microsequencing*, Academic Press, Inc. New York, 1989). A second gel prepared and run in the same manner was stained in a non acetic acid containing stain [9 parts 0.1% Coomassie Blue G250 (Bio-Rad) in 50% (v/v) methanol and 1 part Serva Blue (Serva, Westbury, NY) in distilled water] for 1-2 h. The gel was briefly destained in 20%(v/v) methanol, 3%(v/v) glycerol for 0.5-1 h until the GST-SSP3-5 band was just barely visible. This band was excised from the gel and sent with the electroeluted material to Hazelton Laboratories for use as an antigen in immunizing a New Zealand Rabbit. A total of 1 mg of antigen was used (0.8 mg in gel, 0.2 mg in solution). Test bleeds were provided by Hazelton Laboratories every three weeks. The approximate titer was tested by western blotting of *E. coli* extracts from cells containing the SSP-3-5 gene under the control of the T7 promoter at different dilutions of protein and of serum.

IgG was isolated from the serum using a Protein A Sepharose column. The IgG was coated onto microtiter plates at 5 μg per well. A separate portion of the IgG was biotinylated.

Aqueous extracts from transgenic plants were diluted and loaded into the wells usually starting with a sample containing 1 μg of total protein. The sample was diluted several more times to insure that at least one of the dilutions gave a result that was within the range of a standard curve generated on the same plate. The standard curve was generated using chemically synthesized SSP3-5 protein. The samples were incubated for 1 h at 37° and the plates washed. The biotinylated IgG was then added to the wells. The plate was incubated at 37° for 1 h and washed. Alkaline phosphatase conjugated to streptavidin was added to the wells, incubated at 37° for 1 h and washed. A substrate consisting of 1 mg/mL p nitrophenylphosphate in 1 M diethanolamine was added to the wells and the plates incubated at 37° for 1 h. A 5% EDTA stop solution was added to the wells and the absorbance read at 405 nm minus 650 nm reading. Transgenic soybean seeds contained 0.5 to 2.0% of water extractable protein as SSP3-5.

The remaining half seeds positive for GUS and SSP3-5 protein were planted and grown to maturity in greenhouse conditions. To determine homozygotes for the GUS phenotype, seed from these R1 plants were screened for segregation of GUS activity as above. Plants homozygous for the phaseolin/SSP3-5 gene are then crossed with homozygous transgenic soybeans expressing the *Corynebacterium* dapA gene product or expressing the *Corynebacterium* dapA gene product plus the *E. coli* lysC-M4 gene product.

As an preferred alternative to bringing the chimeric SSP gene and chimeric cordapA gene plus the *E. coli* lysC-M4 gene together via genetic crossing, a single soybean transformation vector carrying all the genes can be constructed from the gene fragments described above and transformed into soybean as described in Example 19.

EXAMPLE 25

Construction of Chimeric Genes for Expression of *Corynebacterium* DHDPS, lys^r-Corn DHDPS, *E. coli*AKIII-M4 and SSP3-5 proteins in the Embryo and Endosperm of Transformed Corn

The following chimeric genes were made for transformation into corn:

globulin 1 promoter/mcts/lysC-M4/NOS 3' region

globulin 1 promoter/mcts/cordapA/NOS 3 region

glutelin 2 promoter/mcts/lysC-M4/NOS 3' region

glutelin 2 promoter/mcts/cordapA/NOS 3' region

globulin 1 promoter/SSP3-5/globulin 1 3' region

glutelin 2 promoter/SSP3-5/10 kD 3' region

globulin 1 promoter/corn lys^r-mutant DHDPS gene/globulin 1 3' region

glutelin 2 promoter/corn lys^r-mutant DHDPS gene/10 kD 3' region

The glutelin 2 promoter was cloned from corn genomic DNA using PCR with primers based on the published sequence [Reina et al. (1990) *Nucleic Acids Res. 18*:6426-6426]. The promoter fragment includes 1020 nucleotides upstream from the ATG translation start codon. An Nco I site was introduced via PCR at the ATG start site to allow for direct translational fusions. A BamH I site was introduced on the 5' end of the promoter. The 1.02 kb BamH I to Nco I promoter fragment was cloned into the BamH I to Nco I sites of the plant expression vector pML63 (see Example 24) replacing the 35S promoter to create vector pML90. This vector contains the glutelin 2 promoter linked to the GUS coding region and the NOS 3'.

The 10 kD zein 3' region was derived from a 10 kD zein gene clone generated by PCR from genomic DNA using oligonucleotide primers based on the published sequence [Kirihara et al. (1988) *Gene 71*:359-370]. The 3' region extends 940 nucleotides from the stop codon. Restriction endonuclease sites for Kpn I, Sma I and Xba I sites were added immediately following the TAG stop codon by oligonucleotide insertion to facilitate cloning. A Sma I to Hind III segment containing the 10 kD 3'region was isolated and ligated into Sma I and Hind III digested pML90 to replace the NOS 3' sequence with the 10 kD 3'region, thus creating plasmid pML103. pML103 contains the glutelin 2 promoter, an Nco I site at the ATG start codon of the GUS gene, Sma I and Xba I sites after the stop codon, and 940 nucleotides of the 10 kD zein 3' sequence.

The globulin 1 promoter and 3' sequences were isolated from a Clontech corn genomic DNA library using oligonucleotide probes based on the published sequence of the globulin 1 gene [Kriz et al. (1989) *Plant Physiol. 91*:636]. The cloned segment includes the promoter fragment extending 1078 nucleotides upstream from the ATG translation start codon, the entire globulin coding sequence including introns and the 3' sequence extending 803 bases from the translational stop. To allow replacement of the globulin 1 coding sequence with other coding sequences an Nco I site was introduced at the ATG start codon, and Kpn I and Xba I sites were introduced following the translational stop codon via PCR to create vector pCC50. There is a second Nco I site within the globulin 1 promoter fragment. The globulin 1 gene cassette is flanked by Hind III sites.

The plant amino acid biosynthetic enzymes are known to be localized in the chloroplasts and therefore are synthesized with a chloroplast targeting signal. Bacterial proteins such as DHDPS and AKIII have no such signal. A chloroplast transit sequence (cts) was therefore fused to the cor<u>dapA</u> and <u>lysC</u>-M4 coding

sequence in the chimeric genes described below. For corn the cts used was based on the cts of the small subunit of ribulose 1,5-bisphosphate carboxylase from corn [Lebrun et al. (1987) *Nucleic Acids Res. 15*:4360] and is designated mcts to distinguish it from the soybean cts. The oligonucleotides SEQ ID NOS:94-99 were synthesized and used as described in Example 6.

To construct the chimeric gene:
globulin 1 promoter/mcts/lysC-M4/NOS 3' region
an Nco I to Hpa I fragment containing the mcts/lysC-M4 coding sequence was isolated from plasmid pBT558 (see Example 6) and inserted into Nco I plus Sma I digested pCC50 creating plasmid pBT663.

To construct the chimeric gene:
globulin 1 promoter/mcts/cordapA/NOS 3 region
an Nco I to Kpn I fragment containing the mcts/ecodapA coding sequence was isolated from plasmid pBT576 (see Example 6) and inserted into Nco I plus Kpn I digested pCC50 creating plasmid pBT662. Then the ecodapA coding sequence was replaced with the cordapA coding sequence as follows. An Afl II to Kpn I fragment containing the distal two thirds of the mcts fused to the cordapA coding sequence was inserted into Afl II to Kpn I digested pBT662 creating plasmid pBT677.

To construct the chimeric gene:
glutelin 2 promoter/mcts/lysC-M4/NOS 3' region
an Nco I to Hpa I fragment containing the mcts/lysC-M4 coding sequence was isolated from plasmid pBT558 (see Example 6) and inserted into Nco I plus Sma I digested pML90 creating plasmid pBT580.

To construct the chimeric gene:
glutelin 2 promoter/mcts/cordapA/NOS 3' region
an Nco I to Kpn I fragment containing the mcts/cordapA coding sequence was isolated from plasmid pBT677 and inserted into Nco I to Kpn I digested pML90, creating plasmid pBT679.

The chimeric genes:
globulin 1 promoter/mcts/lysC-M4/NOS 3' region and
globulin 1 promoter/mcts/cordapA/NOS 3 region
were linked on one plasmid as follows. pBT677 was partially digested with Hind III and full-length linearized plasmid DNA was isolated. A Hind III fragment carrying the globulin 1 promoter/mcts/lysC-M4/NOS 3' region was isolated from pBT663 and ligated to the linearized pBT677 plasmid creating pBT680 (Figure 17).

The chimeric genes:
glutelin 2 promoter/mcts/lysC-M4/NOS 3' region and
glutelin 2 promoter/mcts/cordapA/NOS 3' region
were linked on one plasmid as follows. pBT580 was partially digested with Sal I and full-length linearized plasmid DNA was isolated. A Sal I fragment carrying the glutelin 2 promoter/mcts/cordapA/NOS 3' region was isolated from pBT679 and ligated to the linearized pBT580 plasmid creating pBT681 (Figure 18).

To construct the chimeric gene:
glutelin 2 promoter/SSP3-5/10 kD 3' region
the plasmid pML103 (above) containing the glutelin 2 promoter and 10 kD zein 3' region was cleaved at the Nco I and Sma I sites. The SSP3-5 coding region (Example 22) was isolated as an Nco I to blunt end fragment by cleaving with Xba I followed by filling in the sticky end using Klenow fragment of DNA polymerase, then cleaving with Nco I. The 193 base pair Nco I to blunt end fragment was ligated into the Nco I and Sma I cut pML103 to create pLH104 (Figure 19).

To construct the chimeric gene:
globulin 1 promoter/SSP3-5/globulin 1 3'region
the 193 base pair Nco I and Xba I fragment containing the SSP3-5 coding region (Example 22) was inserted into plasmid pCC50 (above) between the globulin 1 5' and 3' regions creating pLH105 (Figure 20).

The corn DHDPS cDNA gene was cloned and sequenced previously [Frisch et al. (1991) *Mol Gen Genet 228*:287-293]. A mutation that rendered the protein insensitive to feedback inhibition by lysine was introduced into the gene. This mutation is a single nucleotide change that results in a single amino acid substitution in the protein; ala166 is changed to val. The lys^r corn DHDPS gene was obtained from Dr. Burle Gengenbach at the University of Minnesota. An Nco I site was introduced at the translation start codon of the gene and a Kpn I site was introduced immediately following the translation stop codon of the gene via PCR using the following primers:

SEQ ID NO:106: 5'-ATTCCCCATG GTTTCGCCGA CGAAT

SEQ ID NO:107: 5'-CTCTCGGTAC CTAGTACCTA CTGATCAAC

To construct the chimeric gene:
globulin 1 promoter/lys^r corn DHDPS gene/globulin 1 3'region the 1144 base pair Nco I and Kpn I fragment containing the lys^r corn DHDPS gene was inserted into plasmid pCC50 (above) between the globulin 1 5' and 3' regions creating pBT739 (Figure 21).

To construct the chimeric gene:
glutelin 2 promoter/lys^r corn DHDPS gene/10 kD 3' region
the 1144 base pair Nco I and Kpn I fragment containing the lys^r corn DHDPS gene was inserted into a plasmid containing the glutelin 2 promoter and 10 kD zein 3' region creating plasmid pBT756 (Figure 22).

Corn transformations were done as described in Examples 17 and 18 with the following exceptions:

1) Embryogenic cell culture development was as described in Example 17 except the exact culture used for bombardment was designated LH132.5.X, or LH132.6.X.

2) The selectable marker used for these experiments was either the 35S/bar gene from pDETRIC as described in Example 18 or 35S/Ac, a synthetic phosphinothricin-N-acetyltransferase (*pat*) gene under the control of the 35S promoter and 3' terminator/ polyadenylation signal from Cauliflower Mosaic Virus [Eckes et al., (1989) *J Cell Biochem Suppl 13 D*]

3) The bombardment parameters were as described for Example 17 and 18 except that the bombardments were performed as "tribombardments" by co-precipitating 1.5 μg of each of the DNAs (35S/bar or 35S/Ac, pBT681 and pLH104 or 35S/Ac, pbt680 and pLH105) onto the gold particles.

4) Selection of transgenic cell lines was as described for glufosinate selection as in Example 18 except that the tissue was placed on the selection media within 24 h after bombardment.

EXAMPLE 26

Corn Plants Containing Chimeric Genes for Expression of *Corynebacterium* DHDPS and *E. coli*AKIII-M4 or lys^r-Corn DHDPS in the Embryo and Endosperm

Corn was transformed as described in Example 25 with the chimeric genes:

- globulin 1 promoter/mcts/cordapA/NOS 3 region along with or without globulin 1 promoter/mcts/lysC-M4/NOS 3' region; or
- glutelin 2 promoter/mcts/cordapA/NOS 3' region along with or without glutelin 2 promoter/mcts/lysC-M4/NOS 3' region.

Plants regenerated from transformed callus were analyzed for the presence of the intact transgenes via Southern blot or PCR. The plants were either selfed or

outcrossed to an elite line to generate F1 seeds. Six to eight seeds were pooled and assayed for expression of the *Corynebacterium* DHDPS protein and the *E. coli* AKIII-M4 protein by western blot analysis. The free amino acid composition and total amino acid composition of the seeds were determined as described in previous examples.

Expression of the *Corynebacterium* DHDPS protein, driven by either the globulin 1 or glutelin 2 promoter, was observed in the corn seeds (Table 12). Expression of the *E. coli* AKIII-M4 protein, driven by the glutelin promoter was also observed in the corn seeds. Free lysine levels in the seeds increased from about 1.4% of free amino acids in control seeds to 15-27% in seeds of three different transformants expressing *Corynebacterium* DHDPS from the globulin 1 promoter. The increased free lysine, and a high level of saccharopine, indicative of lysine catabolism, were both localized to the embryo in seeds expressing *Corynebacterium* DHDPS from the globulin 1 promoter. No increase in free lysine was observed in seeds expressing *Corynebacterium* DHDPS from the glutelin 2 promoter with or without *E. coli* AKIII-M4. Lysine catabolism is expected to be much greater in the endosperm than the embryo and this probably prevents the accumulation of increased levels of lysine in seeds expressing *Corynebacterium* DHDPS plus *E. coli* AKIII-M4 from the glutelin 2 promoter.

Lysine normally represents about 2.3% of the seed amino acid content. It is therefore apparent from Table 12 that a 130% increase in lysine as a percent of total seed amino acids was found in seeds expressing *Corynebacterium* DHDPS from the globulin 1 promoter.

TABLE 12

TRANSGENIC LINE	PROMOTER	WESTERN CORYNE. DHDPS	WESTERN *E. COLI* AKIII-M4	% LYS OF FREE SEED AMINO ACIDS	% LYS OF TOTAL SEED AMINO ACIDS
1088.1.2 x elite	globulin 1	+	-	15	3.6
1089.4.2 x elite	globulin 1	+	-	21	5.1
1099.2.1 x self	globulin 1	+	-	27	5.3
1090.2.1 x elite	glutelin 2	+	-	1.2	1.7
1092.2.1 x elite	glutelin 2	+	+	1.1	2.2

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: EPELBAUM, SABINE URSULA
FALCO, SAVERIO CARL
MCDEVITT, RAYMOND ERVIN, III

(ii) TITLE OF INVENTION: CHIMERIC GENES AND METHODS FOR INCREASING THE LYSINE CONTENT OF THE SEEDS OF PLANTS

(iii) NUMBER OF SEQUENCES: 132

(iv) CORRESPONDENCE ADDRESS:
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(B) STREET: 1007 MARKET STREET
(C) CITY: WILMINGTON
(D) STATE: DELAWARE
(E) COUNTRY: U.S.A.
(F) ZIP: 19898

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: DISKETTE, 3.50 INCH
(B) COMPUTER: IBM PC COMPATIBLE
(C) OPERATING SYSTEM: MICROSOFT WINDOWS 95
(D) SOFTWARE: MICROSOFT WORD FOR WINDOWS 95 (7.0)

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/824,627
(B) FILING DATE: MARCH 27, 1997

(viii) ATTORNEY/AGENT INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1350 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1350

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
ATG GCT GAA ATT GTT GTC TCC AAA TTT GGC GGT ACC AGC GTA GCT GAT    48
Met Ala Glu Ile Val Val Ser Lys Phe Gly Gly Thr Ser Val Ala Asp
  1               5                  10                  15

TTT GAC GCC ATG AAC CGC AGC GCT GAT ATT GTG CTT TCT GAT GCC AAC    96
Phe Asp Ala Met Asn Arg Ser Ala Asp Ile Val Leu Ser Asp Ala Asn
             20                  25                  30

GTG CGT TTA GTT GTC CTC TCG GCT TCT GCT GGT ATC ACT AAT CTG CTG   144
Val Arg Leu Val Val Leu Ser Ala Ser Ala Gly Ile Thr Asn Leu Leu
         35                  40                  45

GTC GCT TTA GCT GAA GGA CTG GAA CCT GGC GAG CGA TTC GAA AAA CTC   192
Val Ala Leu Ala Glu Gly Leu Glu Pro Gly Glu Arg Phe Glu Lys Leu
     50                  55                  60

GAC GCT ATC CGC AAC ATC CAG TTT GCC ATT CTG GAA CGT CTG CGT TAC   240
Asp Ala Ile Arg Asn Ile Gln Phe Ala Ile Leu Glu Arg Leu Arg Tyr
 65                  70                  75                  80

CCG AAC GTT ATC CGT GAA GAG ATT GAA CGT CTG CTG GAG AAC ATT ACT   288
Pro Asn Val Ile Arg Glu Glu Ile Glu Arg Leu Leu Glu Asn Ile Thr
                 85                  90                  95

GTT CTG GCA GAA GCG GCG GCG CTG GCA ACG TCT CCG GCG CTG ACA GAT   336
Val Leu Ala Glu Ala Ala Ala Leu Ala Thr Ser Pro Ala Leu Thr Asp
            100                 105                 110

GAG CTG GTC AGC CAC GGC GAG CTG ATG TCG ACC CTG CTG TTT GTT GAG   384
Glu Leu Val Ser His Gly Glu Leu Met Ser Thr Leu Leu Phe Val Glu
        115                 120                 125

ATC CTG CGC GAA CGC GAT GTT CAG GCA CAG TGG TTT GAT GTA CGT AAA   432
Ile Leu Arg Glu Arg Asp Val Gln Ala Gln Trp Phe Asp Val Arg Lys
    130                 135                 140

GTG ATG CGT ACC AAC GAC CGA TTT GGT CGT GCA GAG CCA GAT ATA GCC   480
Val Met Arg Thr Asn Asp Arg Phe Gly Arg Ala Glu Pro Asp Ile Ala
145                 150                 155                 160
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GCG CTG GCG GAA CTG GCC GCG CTG CAG CTG CTC CCA CGT CTC AAT GAA   528
Ala Leu Ala Glu Leu Ala Ala Leu Gln Leu Leu Pro Arg Leu Asn Glu
                165                 170                 175

GGC TTA GTG ATC ACC CAG GGA TTT ATC GGT AGC GAA AAT AAA GGT CGT   576
Gly Leu Val Ile Thr Gln Gly Phe Ile Gly Ser Glu Asn Lys Gly Arg
            180                 185                 190

ACA ACG ACG CTT GGC CGT GGA GGC AGC GAT TAT ACG GCA GCC TTG CTG   624
Thr Thr Thr Leu Gly Arg Gly Gly Ser Asp Tyr Thr Ala Ala Leu Leu
        195                 200                 205

GCG GAG GCT TTA CAC GCA TCT CGT GTT GAT ATC TGG ACC GAC GTC CCG   672
Ala Glu Ala Leu His Ala Ser Arg Val Asp Ile Trp Thr Asp Val Pro
    210                 215                 220

GGC ATC TAC ACC ACC GAT CCA CGC GTA GTT TCC GCA GCA AAA CGC ATT   720
Gly Ile Tyr Thr Thr Asp Pro Arg Val Val Ser Ala Ala Lys Arg Ile
225                 230                 235                 240

GAT GAA ATC GCG TTT GCC GAA GCG GCA GAG ATG GCA ACT TTT GGT GCA   768
Asp Glu Ile Ala Phe Ala Glu Ala Ala Glu Met Ala Thr Phe Gly Ala
                245                 250                 255

AAA GTA CTG CAT CCG GCA ACG TTG CTA CCC GCA GTA CGC AGC GAT ATC   816
Lys Val Leu His Pro Ala Thr Leu Leu Pro Ala Val Arg Ser Asp Ile
            260                 265                 270

CCG GTC TTT GTC GGC TCC AGC AAA GAC CCA CGC GCA GGT GGT ACG CTG   864
Pro Val Phe Val Gly Ser Ser Lys Asp Pro Arg Ala Gly Gly Thr Leu
        275                 280                 285

GTG TGC AAT AAA ACT GAA AAT CCG CCG CTG TTC CGC GCT CTG GCG CTT   912
Val Cys Asn Lys Thr Glu Asn Pro Pro Leu Phe Arg Ala Leu Ala Leu
    290                 295                 300

CGT CGC AAT CAG ACT CTG CTC ACT TTG CAC AGC CTG AAT ATG CTG CAT   960
Arg Arg Asn Gln Thr Leu Leu Thr Leu His Ser Leu Asn Met Leu His
305                 310                 315                 320

TCT CGC GGT TTC CTC GCG GAA GTT TTC GGC ATC CTC GCG CGG CAT AAT  1008
Ser Arg Gly Phe Leu Ala Glu Val Phe Gly Ile Leu Ala Arg His Asn
                325                 330                 335

ATT TCG GTA GAC TTA ATC ACC ACG TCA GAA GTG AGC GTG GCA TTA ACC  1056
Ile Ser Val Asp Leu Ile Thr Thr Ser Glu Val Ser Val Ala Leu Thr
            340                 345                 350

CTT GAT ACC ACC GGT TCA ACC TCC ACT GGC GAT ACG TTG CTG ACG CAA  1104
Leu Asp Thr Thr Gly Ser Thr Ser Thr Gly Asp Thr Leu Leu Thr Gln
        355                 360                 365

TCT CTG CTG ATG GAG CTT TCC GCA CTG TGT CGG GTG GAG GTG GAA GAA  1152
Ser Leu Leu Met Glu Leu Ser Ala Leu Cys Arg Val Glu Val Glu Glu
    370                 375                 380
```

```
GGT CTG GCG CTG GTC GCG TTG ATT GGC AAT GAC CTG TCA AAA GCC TGC  1200
Gly Leu Ala Leu Val Ala Leu Ile Gly Asn Asp Leu Ser Lys Ala Cys
385                 390                 395                 400

GCC GTT GGC AAA GAG GTA TTC GGC GTA CTG GAA CCG TTC AAC ATT CGC  1248
Ala Val Gly Lys Glu Val Phe Gly Val Leu Glu Pro Phe Asn Ile Arg
                405                 410                 415

ATG ATT TGT TAT GGC GCA TCC AGC CAT AAC CTG TGC TTC CTG GTG CCC  1296
Met Ile Cys Tyr Gly Ala Ser Ser His Asn Leu Cys Phe Leu Val Pro
            420                 425                 430

GGC GAA GAT GCC GAG CAG GTG GTG CAA AAA CTG CAT AGT AAT TTG TTT  1344
Gly Glu Asp Ala Glu Gln Val Val Gln Lys Leu His Ser Asn Leu Phe
        435                 440                 445

GAG TAA                                                         1350
Glu  *
    450
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GATCCATGGC TGAAATTGTT GTCTCCAAAT TTGGCG 36

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GTACCGCCAA ATTTGGAGAC AACAATTTCA GCCATG 36

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CCCGGGCCAT GGCTACAGGT TTAACAGCTA AGACCGGAGT AGAGCACT 48

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GATATCGAAT TCTCATTATA GAACTCCAGC TTTTTTC 37

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 917 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 3..911

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
CC ATG GCT ACA GGT TTA ACA GCT AAG ACC GGA GTA GAG CAC TTC GGC    47
   Met Ala Thr Gly Leu Thr Ala Lys Thr Gly Val Glu His Phe Gly
     1               5                  10                  15

ACC GTT GGA GTA GCA ATG GTT ACT CCA TTC ACG GAA TCC GGA GAC ATC    95
Thr Val Gly Val Ala Met Val Thr Pro Phe Thr Glu Ser Gly Asp Ile
                20                  25                  30

GAT ATC GCT GCT GGC CGC GAA GTC GCG GCT TAT TTG GTT GAT AAG GGC   143
Asp Ile Ala Ala Gly Arg Glu Val Ala Ala Tyr Leu Val Asp Lys Gly
            35                  40                  45

TTG GAT TCT TTG GTT CTC GCG GGC ACC ACT GGT GAA TCC CCA ACG ACA   191
Leu Asp Ser Leu Val Leu Ala Gly Thr Thr Gly Glu Ser Pro Thr Thr
        50                  55                  60

ACC GCC GCT GAA AAA CTA GAA CTG CTC AAG GCC GTT CGT GAG GAA GTT   239
Thr Ala Ala Glu Lys Leu Glu Leu Leu Lys Ala Val Arg Glu Glu Val
    65                  70                  75

GGG GAT CGG GCG AAG CTC ATC GCC GGT GTC GGA ACC AAC AAC ACG CGG   287
Gly Asp Arg Ala Lys Leu Ile Ala Gly Val Gly Thr Asn Asn Thr Arg
80                  85                  90                  95
```

```
ACA TCT GTG GAA CTT GCG GAA GCT GCT GCT TCT GCT GGC GCA GAC GGC  335
Thr Ser Val Glu Leu Ala Glu Ala Ala Ala Ser Ala Gly Ala Asp Gly
                100                 105                 110

CTT TTA GTT GTA ACT CCT TAT TAC TCC AAG CCG AGC CAA GAG GGA TTG  383
Leu Leu Val Val Thr Pro Tyr Tyr Ser Lys Pro Ser Gln Glu Gly Leu
            115                 120                 125

CTG GCG CAC TTC GGT GCA ATT GCT GCA GCA ACA GAG GTT CCA ATT TGT  431
Leu Ala His Phe Gly Ala Ile Ala Ala Ala Thr Glu Val Pro Ile Cys
        130                 135                 140

CTC TAT GAC ATT CCT GGT CGG TCA GGT ATT CCA ATT GAG TCT GAT ACC  479
Leu Tyr Asp Ile Pro Gly Arg Ser Gly Ile Pro Ile Glu Ser Asp Thr
    145                 150                 155

ATG AGA CGC CTG AGT GAA TTA CCT ACG ATT TTG GCG GTC AAG GAC GCC  527
Met Arg Arg Leu Ser Glu Leu Pro Thr Ile Leu Ala Val Lys Asp Ala
160                 165                 170                 175

AAG GGT GAC CTC GTT GCA GCC ACG TCA TTG ATC AAA GAA ACG GGA CTT  575
Lys Gly Asp Leu Val Ala Ala Thr Ser Leu Ile Lys Glu Thr Gly Leu
                180                 185                 190

GCC TGG TAT TCA GGC GAT GAC CCA CTA AAC CTT GTT TGG CTT GCT TTG  623
Ala Trp Tyr Ser Gly Asp Asp Pro Leu Asn Leu Val Trp Leu Ala Leu
            195                 200                 205

GGC GGA TCA GGT TTC ATT TCC GTA ATT GGA CAT GCA GCC CCC ACA GCA  671
Gly Gly Ser Gly Phe Ile Ser Val Ile Gly His Ala Ala Pro Thr Ala
        210                 215                 220

TTA CGT GAG TTG TAC ACA AGC TTC GAG GAA GGC GAC CTC GTC CGT GCG  719
Leu Arg Glu Leu Tyr Thr Ser Phe Glu Glu Gly Asp Leu Val Arg Ala
    225                 230                 235

CGG GAA ATC AAC GCC AAA CTA TCA CCG CTG GTA GCT GCC CAA GGT CGC  767
Arg Glu Ile Asn Ala Lys Leu Ser Pro Leu Val Ala Ala Gln Gly Arg
240                 245                 250                 255

TTG GGT GGA GTC AGC TTG GCA AAA GCT GCT CTG CGT CTG CAG GGC ATC  815
Leu Gly Gly Val Ser Leu Ala Lys Ala Ala Leu Arg Leu Gln Gly Ile
                260                 265                 270

AAC GTA GGA GAT CCT CGA CTT CCA ATT ATG GCT CCA AAT GAG CAG GAA  863
Asn Val Gly Asp Pro Arg Leu Pro Ile Met Ala Pro Asn Glu Gln Glu
            275                 280                 285

CTT GAG GCT CTC CGA GAA GAC ATG AAA AAA GCT GGA GTT CTA TAA TGAGAATTC  918
Leu Glu Ala Leu Arg Glu Asp Met Lys Lys Ala Gly Val Leu  *
        290                 295                 300
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CTTCCCGTGA CCATGGGCCA TC 22

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 75 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CATGGCTGGC TTCCCCACGA GGAAGACCAA CAATGACATT ACCTCCATTG CTAGCAACGG 60

TGGAAGAGTA CAATG 75

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 75 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CATGCATTGT ACTCTTCCAC CGTTGCTAGC AATGGAGGTA ATGTCATTGT TGGTCTTCCT 60

CGTGGGGAAG CCAGC 75

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 90 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CATGGCTTCC TCAATGATCT CCTCCCCAGC TGTTACCACC GTCAACCGTG CCGGTGCCGG 60

CATGGTTGCT CCATTCACCG GCCTCAAAAG 90

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 90 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CATGCTTTTG AGGCCGGTGA ATGGAGCAAC CATGCCGGCA CCGGCACGGT TGACGGTGGT 60

AACAGCTGGG GAGGAGATCA TTGAGGAAGC 90

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CCGGTTTGCT GTAATAGGTA CCA 23

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

AGCTTGGTAC CTATTACAGC AAACCGGCAT G 31

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GCTTCCTCAA TGATCTCCTC CCCAGCT 27

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CATTGTACTC TTCCACCGTT GCTAGCAA 28

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..20
(D) OTHER INFORMATION: /product= "synthetic oligonucleotide" /standard_name= "SM 70"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CTGACTCGCT GCGCTCGGTC 20

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..24
(D) OTHER INFORMATION: /product= "synthetic oligonucleotide" /standard_name= "SM 71"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

TATTTTCTCC TTACGCATCT GTGC 24

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..27
(D) OTHER INFORMATION: /product= "synthetic oligonucleotide" /standard_name= "SM 78"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

TTCATCGATA GGCGACCACA CCCGTCC 27

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..27
(D) OTHER INFORMATION: /product= "synthetic oligonucleotide" /standard_name= "SM 79"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

AATATCGATG CCACGATGCG TCCGGCG 27

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 55 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..55

(D) OTHER INFORMATION: /product= "synthetic oligonucleotide" /standard_name= "SM 81"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

CATGGAGGAG AAGATGAAGG CGATGGAAGA GAAGATGAAG GCGTGATAGG TACCG 55

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 55 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..55
(D) OTHER INFORMATION: /product= "synthetic oligonucleotide" /standard_name= "SM 80"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

AATTCGGTAC CTATCACGCC TTCATCTTCT CTTCCATCGC CTTCATCTTC TCCTC 55

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..14
(D) OTHER INFORMATION: /label= name /note= "base gene [(SSP5)2]"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Met Glu Glu Lys Met Lys Ala Met Glu Glu Lys Met Lys Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..21
(D) OTHER INFORMATION: /product= "synthetic oligonucleotide" /standard_name= "SM 84"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GATGGAGGAG AAGATGAAGG C 21

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..21
(D) OTHER INFORMATION: /product= "synthetic oligonucleotide" /standard_name= "SM 85"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

ATCGCCTTCA TCTTCTCCTC C 21

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..21
(D) OTHER INFORMATION: /product= "synthetic oligonucleotide" /standard_name= "SM 82"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GATGGAGGAG AAGCTGAAGG C 21

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..21
(D) OTHER INFORMATION: /product= "synthetic oligonucleotide" /standard_name= "SM 83"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

ATCGCCTTCA GCTTCTCCTC C 21

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Met Glu Glu Lys Leu Lys Ala
1 5

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Met Glu Glu Lys Met Lys Ala
1 5

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 160 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(B) STRAIN: E. coli
(G) CELL TYPE: DH5 alpha

(vii) IMMEDIATE SOURCE:
(B) CLONE: C15

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2..151
(D) OTHER INFORMATION: /function= "synthetic storage protein"
/product= "protein"
/gene= "ssp"
/standard_name= "5.7.7.7.7.7.5"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

```
C ATG GAG GAG AAG ATG AAG GCG ATG GAG GAG AAG CTG AAG GCG ATG     46
  Met Glu Glu Lys Met Lys Ala Met Glu Glu Lys Leu Lys Ala Met
    1               5                  10                  15

GAG GAG AAG CTG AAG GCG ATG GAG GAG AAG CTG AAG GCG ATG GAG GAG  94
Glu Glu Lys Leu Lys Ala Met Glu Glu Lys Leu Lys Ala Met Glu Glu
                20                  25                  30

AAG CTG AAG GCG ATG GAG GAG AAG CTG AAG GCG ATG GAA GAG AAG ATG 142
Lys Leu Lys Ala Met Glu Glu Lys Leu Lys Ala Met Glu Glu Lys Met
            35                  40                  45

AAG GCG TGATAGGTAC CG                                          160
Lys Ala
        50
```

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 49 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

```
Met Glu Glu Lys Met Lys Ala Met Glu Glu Lys Leu Lys Ala Met Glu
  1               5                  10                  15
```

Glu Lys Leu Lys Ala Met Glu Glu Lys Leu Lys Ala Met Glu Glu Lys
20 25 30

Leu Lys Ala Met Glu Glu Lys Leu Lys Ala Met Glu Glu Lys Met Lys
35 40 45

Ala

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 160 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(B) STRAIN: E. coli
(G) CELL TYPE: DH5 alpha

(vii) IMMEDIATE SOURCE:
(B) CLONE: C20

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2..151
(D) OTHER INFORMATION: /function= "synthetic storage protein"
/product= "protein"
/gene= "ssp"
/standard_name= "5.7.7.7.7.7.5"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

```
C ATG GAG GAG AAG ATG AAG GCG ATG GAG GAG AAG CTG AAG GCG ATG      46
  Met Glu Glu Lys Met Lys Ala Met Glu Glu Lys Leu Lys Ala Met
    1               5                  10                  15

GAG GAG AAG CTG AAG GCG ATG GAG GAG AAG CTG AAG GCG ATG GAG GAG    94
Glu Glu Lys Leu Lys Ala Met Glu Glu Lys Leu Lys Ala Met Glu Glu
                20                  25                  30

AAG CTG AAG GCG ATG GAG GAG AAG CTG AAG GCG ATG GAA GAG AAG ATG  142
Lys Leu Lys Ala Met Glu Glu Lys Leu Lys Ala Met Glu Glu Lys Met
            35                  40                  45

AAG GCG TGATAGGTAC CG                                           160
Lys Ala
        50
```

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 49 amino acids
(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

```
Met Glu Glu Lys Met Lys Ala Met Glu Glu Lys Leu Lys Ala Met Glu
 1               5                  10                  15

Glu Lys Leu Lys Ala Met Glu Glu Lys Leu Lys Ala Met Glu Glu Lys
            20                  25                  30

Leu Lys Ala Met Glu Glu Lys Leu Lys Ala Met Glu Glu Lys Met Lys
        35                  40                  45

Ala
```

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 139 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(B) STRAIN: E. coli
(G) CELL TYPE: DH5 alpha

(vii) IMMEDIATE SOURCE:
(B) CLONE: C30

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2..130
(D) OTHER INFORMATION: /function= "synthetic storage protein"
/product= "protein"
/gene= "ssp"
/standard_name= "5.7.7.7.7.5"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

```
C ATG GAG GAG AAG ATG AAG GCG ATG GAG GAG AAG CTG AAG GCG ATG      46
  Met Glu Glu Lys Met Lys Ala Met Glu Glu Lys Leu Lys Ala Met
   1               5                  10                  15

GAG GAG AAG CTG AAG GCG ATG GAG GAG AAG CTG AAG GCG ATG GAG GAG    94
Glu Glu Lys Leu Lys Ala Met Glu Glu Lys Leu Lys Ala Met Glu Glu
            20                  25                  30

AAG CTG AAG GCG ATG GAA GAG AAG ATG AAG GCG TGATAGGTAC CG         139
Lys Leu Lys Ala Met Glu Glu Lys Met Lys Ala
        35                  40
```

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

```
Met Glu Glu Lys Met Lys Ala Met Glu Glu Lys Leu Lys Ala Met Glu
 1               5                  10                  15

Glu Lys Leu Lys Ala Met Glu Glu Lys Leu Lys Ala Met Glu Glu Lys
            20                  25                  30

Leu Lys Ala Met Glu Glu Lys Met Lys Ala
        35                  40
```

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 97 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(B) STRAIN: E. coli
(G) CELL TYPE: DH5 alpha

(vii) IMMEDIATE SOURCE:
(B) CLONE: D16

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2..88
(D) OTHER INFORMATION: /function= "synthetic storage protein"
/product= "protein"
/gene= "ssp"
/standard_name= "5.5.5.5"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

```
C ATG GAG GAG AAG ATG AAG GCG ATG GAG GAG AAG ATG AAG GCG ATG   46
  Met Glu Glu Lys Met Lys Ala Met Glu Glu Lys Met Lys Ala Met
    1               5                  10                  15

GAG GAG AAG ATG AAG GCG ATG GAA GAG AAG ATG AAG GCG TGATAGGTAC  95
Glu Glu Lys Met Lys Ala Met Glu Glu Lys Met Lys Ala
                20                  25

CG                                                             97
```

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

```
Met Glu Glu Lys Met Lys Ala Met Glu Glu Lys Met Lys Ala Met Glu
 1               5                  10                  15

Glu Lys Met Lys Ala Met Glu Glu Lys Met Lys Ala
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 118 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(B) STRAIN: E. coli
(G) CELL TYPE: DH5 alpha

(vii) IMMEDIATE SOURCE:
(B) CLONE: D20

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2..109
(D) OTHER INFORMATION: /function= "synthetic storage protein"
/product= "protein"
/gene= "ssp"
/standard_name= "5.5.5.5.5"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

```
C ATG GAG GAG AAG ATG AAG GCG ATG GAG GAG AAG ATG AAG GCG ATG     46
  Met Glu Glu Lys Met Lys Ala Met Glu Glu Lys Met Lys Ala Met
    1               5                  10                  15

GAG GAG AAG ATG AAG GCG ATG GAG GAG AAG ATG AAG GCG ATG GAA GAG  94
Glu Glu Lys Met Lys Ala Met Glu Glu Lys Met Lys Ala Met Glu Glu
                20                  25                  30

AAG ATG AAG GCG TGATAGGTAC CG                                   118
Lys Met Lys Ala
            35
```

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

```
Met Glu Glu Lys Met Lys Ala Met Glu Glu Lys Met Lys Ala Met Glu
 1               5                  10                  15

Glu Lys Met Lys Ala Met Glu Glu Lys Met Lys Ala Met Glu Glu Lys
            20                  25                  30

Met Lys Ala
        35
```

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 97 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(B) STRAIN: E. coli
(G) CELL TYPE: DH5 alpha

(vii) IMMEDIATE SOURCE:
(B) CLONE: D33

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2..88
(D) OTHER INFORMATION: /function= "synthetic storage protein"
/product= "protein"
/gene= "ssp"
/standard_name= "5.5.5.5"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

```
C ATG GAG GAG AAG ATG AAG GCG ATG GAG GAG AAG ATG AAG GCG ATG   46
  Met Glu Glu Lys Met Lys Ala Met Glu Glu Lys Met Lys Ala Met
   1               5                  10                  15

GAG GAG AAG ATG AAG GCG ATG GAA GAG AAG ATG AAG GCG TGATAGGTAC  95
Glu Glu Lys Met Lys Ala Met Glu Glu Lys Met Lys Ala
                20                  25
```

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Met Glu Glu Lys Met Lys Ala Met Glu Glu Lys Met Lys Ala Met Glu
1 5 10 15

Glu Lys Met Lys Ala Met Glu Glu Lys Met Lys Ala
20 25

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..21
(D) OTHER INFORMATION: /product= "synthetic oligonucleotide" /standard_name= "SM 86"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

GATGGAGGAG AAGCTGAAGA A 21

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..21
(D) OTHER INFORMATION: /product= "synthetic oligonucleotide" /standard_name= "SM 87"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

ATCTTCTTCA GCTTCTCCTC C 21

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..21
(D) OTHER INFORMATION: /product= "synthetic oligonucleotide" /standard_name= "SM 88"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

GATGGAGGAG AAGCTGAAGT G 21

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..21
(D) OTHER INFORMATION: /product= "synthetic oligonucleotide" /standard_name= "SM 89"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

ATCCACTTCA GCTTCTCCTC C 21

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..21
(D) OTHER INFORMATION: /product= "synthetic oligonucleotide" /standard_name= "SM 90"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

GATGGAGGAG AAGATGAAGA A 21

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..21
(D) OTHER INFORMATION: /product= "synthetic oligonucleotide" /standard_name= "SM 91"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

ATCTTCTTCA TCTTCTCCTC C 21

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..21
(D) OTHER INFORMATION: /product= "synthetic oligonucleotide" /standard_name= "SM 92"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

GATGGAGGAG AAGATGAAGT G 21

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..21
(D) OTHER INFORMATION: /product= "synthetic oligonucleotide" /standard_name= "SM 93"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

ATCCACTTCA TCTTCTCCTC C 21

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

Met Glu Glu Lys Leu Lys Lys
1 5

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

Met Glu Glu Lys Leu Lys Trp
1 5

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid

(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

Met Glu Glu Lys Met Lys Lys
1 5

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

Met Glu Glu Lys Met Lys Trp
1 5

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 160 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(B) STRAIN: E. coli
(G) CELL TYPE: DH5 alpha

(vii) IMMEDIATE SOURCE:
(B) CLONE: 82-4

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2..151
(D) OTHER INFORMATION: /function= "synthetic storage protein
/product= "protein"
/gene= "ssp"
/standard_name= "7.7.7.7.7.7.5"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

C ATG GAG GAG AAG CTG AAG GCG ATG GAG GAG AAG CTG AAG GCG ATG 46
Met Glu Glu Lys Leu Lys Ala Met Glu Glu Lys Leu Lys Ala Met
1 5 10 15

```
GAG GAG AAG CTG AAG GCG ATG GAG GAG AAG CTG AAG GCG ATG GAG GAG  94
Glu Glu Lys Leu Lys Ala Met Glu Glu Lys Leu Lys Ala Met Glu Glu
                20                  25                  30

AAG CTG AAG GCG ATG GAG GAG AAG CTG AAG GCG ATG GAA GAG AAG ATG 142
Lys Leu Lys Ala Met Glu Glu Lys Leu Lys Ala Met Glu Glu Lys Met
            35                  40                  45

AAG GCG TGATAGGTAC CG                                          160
Lys Ala
        50
```

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 49 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

```
Met Glu Glu Lys Leu Lys Ala Met Glu Glu Lys Leu Lys Ala Met Glu
 1               5                  10                  15

Glu Lys Leu Lys Ala Met Glu Glu Lys Leu Lys Ala Met Glu Glu Lys
            20                  25                  30

Leu Lys Ala Met Glu Glu Lys Leu Lys Ala Met Glu Glu Lys Met Lys
        35                  40                  45

Ala
```

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 97 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(B) STRAIN: E. coli
(G) CELL TYPE: DH5 alpha

(vii) IMMEDIATE SOURCE:
(B) CLONE: 84-H3

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2..88

(D) OTHER INFORMATION: /function= "synthetic storage protein /product= "protein" /gene= "ssp" /standard_name= "5.5.5.5"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

```
C ATG GAG GAG AAG ATG AAG GCG ATG GAG GAG AAG ATG AAG GCG ATG   46
  Met Glu Glu Lys Met Lys Ala Met Glu Glu Lys Met Lys Ala Met
    1               5                  10                  15

GAG GAG AAG ATG AAG GCG ATG GAA GAG AAG ATG AAG GCG TGATAGGTAC  95
Glu Glu Lys Met Lys Ala Met Glu Glu Lys Met Lys Ala
                20                  25

CG                                                              97
```

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

```
Met Glu Glu Lys Met Lys Ala Met Glu Glu Lys Met Lys Ala Met Glu
  1               5                  10                  15

Glu Lys Met Lys Ala Met Glu Glu Lys Met Lys Ala
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 97 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(B) STRAIN: E. coli
(G) CELL TYPE: DH5 alpha

(vii) IMMEDIATE SOURCE:
(B) CLONE: 86-H23

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2..88

(D) OTHER INFORMATION: /function= "synthetic storage protein /product= "protein" /gene= "ssp" /standard_name= "5.8.8.5"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

```
C ATG GAG GAG AAG ATG AAG GCG ATG GAG GAG AAG CTG AAG AAG ATG   46
  Met Glu Glu Lys Met Lys Ala Met Glu Glu Lys Leu Lys Lys Met
    1               5                  10                  15

GAG GAG AAG CTG AAG AAG ATG GAA GAG AAG ATG AAG GCG TGATAGGTAC  95
Glu Glu Lys Leu Lys Lys Met Glu Glu Lys Met Lys Ala
                20                  25

CG                                                              97
```

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

```
Met Glu Glu Lys Met Lys Ala Met Glu Glu Lys Leu Lys Lys Met Glu
  1               5                  10                  15

Glu Lys Leu Lys Lys Met Glu Glu Lys Met Lys Ala
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 112 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(B) STRAIN: E. coli
(G) CELL TYPE: DH5 alpha

(vii) IMMEDIATE SOURCE:
(B) CLONE: 88-2

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2..103

(D) OTHER INFORMATION: /function= "synthetic storage protein /product= "protein" /gene= "ssp" /standard_name= "5.9.9.9.5"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

```
C ATG GAG GAG AAG ATG AAG GCG AAG AAG CTG AAG TGG ATG GAG GAG   46
  Met Glu Glu Lys Met Lys Ala Lys Lys Leu Lys Trp Met Glu Glu
    1               5                  10                  15

AAG CTG AAG TGG ATG GAG GAG AAG CTG AAG TGG ATG GAA GAG AAG ATG 94
Lys Leu Lys Trp Met Glu Glu Lys Leu Lys Trp Met Glu Glu Lys Met
                 20                  25                  30

AAG GCG TGATAGGTAC CG                                         112
Lys Ala
```

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

```
Met Glu Glu Lys Met Lys Ala Lys Lys Leu Lys Trp Met Glu Glu Lys
  1               5                  10                  15

Leu Lys Trp Met Glu Glu Lys Leu Lys Trp Met Glu Glu Lys Met Lys
             20                  25                  30

Ala
```

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 118 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(B) STRAIN: E. coli
(G) CELL TYPE: DH5 alpha

(vii) IMMEDIATE SOURCE:
(B) CLONE: 90-H8

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2..109
(D) OTHER INFORMATION: /function= "synthetic storage protein
/product= "protein"
/gene= "ssp"
/standard_name= "5.10.10.10.5"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

```
C ATG GAG GAG AAG ATG AAG GCG ATG GAG GAG AAG ATG AAG AAG ATG    46
  Met Glu Glu Lys Met Lys Ala Met Glu Glu Lys Met Lys Lys Met
    1               5                  10                  15

GAG GAG AAG ATG AAG AAG ATG GAG GAG AAG ATG AAG AAG ATG GAA GAG 94
Glu Glu Lys Met Lys Lys Met Glu Glu Lys Met Lys Lys Met Glu Glu
                20                  25                  30

AAG ATG AAG GCG TGATAGGTAC CG                                   118
Lys Met Lys Ala
            35
```

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

```
Met Glu Glu Lys Met Lys Ala Met Glu Glu Lys Met Lys Lys Met Glu
  1               5                  10                  15

Glu Lys Met Lys Lys Met Glu Glu Lys Met Lys Lys Met Glu Glu Lys
            20                  25                  30

Met Lys Ala
        35
```

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 97 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(B) STRAIN: E. coli
(G) CELL TYPE: DH5 alpha

(vii) IMMEDIATE SOURCE:
(B) CLONE: 92-2

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2..88
(D) OTHER INFORMATION: /function= "synthetic storage protein
/product= "protein"
/gene= "ssp"
/standard_name= "5.11.11.5"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

```
C ATG GAG GAG AAG ATG AAG GCG ATG GAG GAG AAG ATG AAG TGG ATG     46
  Met Glu Glu Lys Met Lys Ala Met Glu Glu Lys Met Lys Trp Met
    1               5                  10                  15

GAG GAG AAG ATG AAG TGG ATG GAA GAG AAG ATG AAG GCG TGATAGGTAC   95
Glu Glu Lys Met Lys Trp Met Glu Glu Lys Met Lys Ala
                20                  25

CG                                                              97
```

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

```
Met Glu Glu Lys Met Lys Ala Met Glu Glu Lys Met Lys Trp Met Glu
  1               5                  10                  15

Glu Lys Met Lys Trp Met Glu Glu Lys Met Lys Ala
             20                  25
```

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 84 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..84

(D) OTHER INFORMATION: /product= "synthetic oligonucleotide" /standard_name= "SM 96"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

GATGGAGGAA AAGATGAAGG CGATGGAGGA GAAAATGAAA GCTATGGAGG AAAAGATGAA 60

AGCGATGGAG GAGAAAATGA AGGC 84

(2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 84 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..84
(D) OTHER INFORMATION: /product= "synthetic oligonucleotide" /standard_name= "SM 97"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

ATCGCCTTCA TTTTCTCCTC CATCGCTTTC ATCTTTTCCT CCATAGCTTT CATTTTCTCC 60

TCCATCGCCT TCATCTTTTC CTCC 84

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..28
(D) OTHER INFORMATION: /label= name /note= "(SSP 5)4"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

Met Glu Glu Lys Met Lys Ala Met Glu Glu Lys Met Lys Ala Met Glu
1 5 10 15

Glu Lys Met Lys Ala Met Glu Glu Lys Met Lys Ala
20 25

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 84 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..84
(D) OTHER INFORMATION: /product= "synthetic oligonucleotide" /standard_name= "SM 98"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

GATGGAGGAA AAGCTGAAAG CGATGGAGGA GAAACTCAAG GCTATGGAAG AAAAGCTTAA 60

AGCGATGGAG GAGAAACTGA AGGC 84

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 84 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..84
(D) OTHER INFORMATION: /product= "synthetic oligonucleotide" /standard_name= "SM 99"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

ATCGCCTTCA GTTTCTCCTC CTACGCTTTA AGCTTTTCTT CCATAGCCTT GAGTTTCTCC 60

TCCATCGCTT TCAGCTTTTC CTCC 84

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..28
(D) OTHER INFORMATION: /label= name
/note= "(SSP 7)4"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

Met Glu Glu Lys Leu Lys Ala Met Glu Glu Lys Leu Lys Ala Met Glu
1 5 10 15

Glu Lys Leu Lys Ala Met Glu Glu Lys Leu Lys Ala
20 25

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 84 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..84
(D) OTHER INFORMATION: /product= "synthetic
oligonucleotide"
/standard_name= "SM
100"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

GATGGAGGAA AAGCTTAAGA AGATGGAAGA AAAGCTGAAA TGGATGGAGG AGAAACTCAA 60

AAAGATGGAG GAAAAGCTTA AATG 84

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 84 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..84
(D) OTHER INFORMATION: /product= "synthetic
oligonucleotide"
/standard_name= "SM
101"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

ATCCATTTAA GCTTTTCCTC CTACTTTTTG AGTTTCTCCT CCATCCATTT CAGCTTTTCT 60

TCCATCTTCT TAAGCTTTTC CTCC 84

(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

Met Glu Glu Lys Leu Lys Lys Met Glu Glu Lys Leu Lys Trp Met Glu
1 5 10 15

Glu Lys Leu Lys Lys Met Glu Glu Lys Leu Lys Trp
20 25

(2) INFORMATION FOR SEQ ID NO:74:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 243 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(B) STRAIN: E. coli
(G) CELL TYPE: DH5 alpha

(vii) IMMEDIATE SOURCE:
(B) CLONE: 2-9

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2..235
(D) OTHER INFORMATION: /function= "synthetic
storage protein
/product= "protein"
/gene= "ssp"
/standard_name=
"7.7.7.7.7.7.8.9.8.9.5"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

C ATG GAG GAG AAG CTG AAG GCG ATG GAG GAG AAG CTG AAG GCG ATG 46
Met Glu Glu Lys Leu Lys Ala Met Glu Glu Lys Leu Lys Ala Met
1 5 10 15

```
GAG GAG AAG CTG AAG GCG ATG GAG GAG AAG CTG AAG GCG ATG GAG GAG      94
Glu Glu Lys Leu Lys Ala Met Glu Glu Lys Leu Lys Ala Met Glu Glu
                20                  25                  30

AAG CTG AAG GCG ATG GAG GAG AAG CTG AAG GCG ATG GAG GAA AAG CTT     142
Lys Leu Lys Ala Met Glu Glu Lys Leu Lys Ala Met Glu Glu Lys Leu
            35                  40                  45

AAG AAG ATG GAA GAA AAG CTG AAA TGG ATG GAG GAG AAA CTC AAA AAG     190
Lys Lys Met Glu Glu Lys Leu Lys Trp Met Glu Glu Lys Leu Lys Lys
        50                  55                  60

ATG GAG GAA AAG CTT AAA TGG ATG GAA GAG AAG ATG AAG GCG TGATAGGTAC 242
Met Glu Glu Lys Leu Lys Trp Met Glu Glu Lys Met Lys Ala
    65                  70                  75

C                                                                   243
```

(2) INFORMATION FOR SEQ ID NO:75:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

```
Met Glu Glu Lys Leu Lys Ala Met Glu Glu Lys Leu Lys Ala Met Glu
  1               5                  10                  15

Glu Lys Leu Lys Ala Met Glu Glu Lys Leu Lys Ala Met Glu Glu Lys
            20                  25                  30

Leu Lys Ala Met Glu Glu Lys Leu Lys Ala Met Glu Glu Lys Leu Lys
        35                  40                  45

Lys Met Glu Glu Lys Leu Lys Trp Met Glu Glu Lys Leu Lys Lys Met
    50                  55                  60

Glu Glu Lys Leu Lys Trp Met Glu Glu Lys Met Lys Ala
65                  70                  75
```

(2) INFORMATION FOR SEQ ID NO:76:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 175 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(B) STRAIN: E. coli
(G) CELL TYPE: DH5 alpha

(vii) IMMEDIATE SOURCE:
(B) CLONE: 5-1

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2..172

(D) OTHER INFORMATION: /function= "synthetic storage protein
/product= "protein"
/gene= "ssp"
/standard_name=
"5.5.5.7.7.7.7.5"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

```
C ATG GAG GAG AAG ATG AAG GCG ATG GAG GAG AAG ATG AAG GCG ATG     46
  Met Glu Glu Lys Met Lys Ala Met Glu Glu Lys Met Lys Ala Met
    1               5                  10                  15

GAG GAG AAG ATG AAG GCG ATG GAG GAA AAG CTG AAA GCG ATG GAG GAG  94
Glu Glu Lys Met Lys Ala Met Glu Glu Lys Leu Lys Ala Met Glu Glu
                20                  25                  30

AAA CTC AAG GCT ATG GAA GAA AAG CTT AAA GCG ATG GAG GAG AAA CTG 142
Lys Leu Lys Ala Met Glu Glu Lys Leu Lys Ala Met Glu Glu Lys Leu
            35                  40                  45

AAG GCC ATG GAA GAG AAG ATG AAG GCG TGATAG                      179
Lys Ala Met Glu Glu Lys Met Lys Ala
        50                  55
```

(2) INFORMATION FOR SEQ ID NO:77:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 56 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

```
Met Glu Glu Lys Met Lys Ala Met Glu Glu Lys Met Lys Ala Met Glu
  1               5                  10                  15

Glu Lys Met Lys Ala Met Glu Glu Lys Leu Lys Ala Met Glu Glu Lys
            20                  25                  30

Leu Lys Ala Met Glu Glu Lys Leu Lys Ala Met Glu Glu Lys Leu Lys
        35                  40                  45

Ala Met Glu Glu Lys Met Lys Ala
    50                  55
```

(2) INFORMATION FOR SEQ ID NO:78:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 187 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(B) STRAIN: E. coli
(G) CELL TYPE: DH5 alpha

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 3..173
(D) OTHER INFORMATION: /function= "synthetic storage protein
/product= "protein"
/gene= "ssp"
/standard_name= "SSP-3-5"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

```
CC ATG GAG GAG AAG CTG AAG GCG ATG GAG GAG AAG CTG AAG GCG ATG    47
   Met Glu Glu Lys Leu Lys Ala Met Glu Glu Lys Leu Lys Ala Met
     1               5                  10                  15

GAG GAG AAG CTG AAG GCG ATG GAG GAG AAG CTG AAG GCG ATG GAG GAG   95
Glu Glu Lys Leu Lys Ala Met Glu Glu Lys Leu Lys Ala Met Glu Glu
                 20                  25                  30

AAG CTG AAG GCG ATG GAG GAG AAG CTG AAG GCG ATG GAG GAA AAG ATG  143
Lys Leu Lys Ala Met Glu Glu Lys Leu Lys Ala Met Glu Glu Lys Met
             35                  40                  45

AAG GCG ATG GAA GAG AAG ATG AAG GCG TGATAGGTAC CGAATTC           187
Lys Ala Met Glu Glu Lys Met Lys Ala
         50                  55
```

(2) INFORMATION FOR SEQ ID NO:79:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 56 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

```
Met Glu Glu Lys Leu Lys Ala Met Glu Glu Lys Leu Lys Ala Met Glu
  1               5                  10                  15

Glu Lys Leu Lys Ala Met Glu Glu Lys Leu Lys Ala Met Glu Glu Lys
             20                  25                  30
```

Leu Lys Ala Met Glu Glu Lys Leu Lys Ala Met Glu Glu Lys Met Lys
35 40 45

Ala Met Glu Glu Lys Met Lys Ala
50 55

(2) INFORMATION FOR SEQ ID NO:80:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 61 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..61
(D) OTHER INFORMATION: /product= "synthetic oligonucleotide" /standard_name= "SM 107"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

CATGGAGGAG AAGATGAAAA AGCTCGAAGA GAAGATGAAG GTCATGAAGT GATAGGTACC 60

G 61

(2) INFORMATION FOR SEQ ID NO:81:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 61 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..61
(D) OTHER INFORMATION: /product= "synthetic ligonucleotide" /standard_name= "SM 106"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

AATTCGGTAC CTATCACTTC ATGACCTTCA TCTTCTCTTC GAGCTTTTTC ATCTTCTCCT 60

C 61

(2) INFORMATION FOR SEQ ID NO:82:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..16
(D) OTHER INFORMATION: /label= name
/note= "pSK34 base gene"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

Met Glu Glu Lys Met Lys Lys Leu Glu Glu Lys Met Lys Val Met Lys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:83:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 63 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..63
(D) OTHER INFORMATION: /product= "synthetic oligonucleotide"
/standard_name= "SM 110"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

GCTGGAAGAA AAGATGAAGG CTATGGAGGA CAAGATGAAA TGGCTTGAGG AAAAGATGAA 60

GAA 63

(2) INFORMATION FOR SEQ ID NO:84:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 63 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..63
(D) OTHER INFORMATION: /product= "synthetic oligonucleotide" /standard_name= "SM 111"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

AGCTTCTTCA TCTTTTCCTC AAGCCATTTC ATCTTGTCCT CCATAGCCTT CATCTTTTCT 60

TCC 63

(2) INFORMATION FOR SEQ ID NO:85:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

Met Glu Glu Lys Met Lys Lys Leu Glu Glu Lys Met Lys Ala Met Glu
1 5 10 15

Asp Lys Met Lys Trp Leu Glu Glu Lys Met Lys Lys Leu Glu Glu Lys
20 25 30

Met Lys Val Met Lys
35

(2) INFORMATION FOR SEQ ID NO:86:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

Met Glu Glu Lys Met Lys Lys Leu Glu Glu Lys Met Lys Ala Met Glu
1 5 10 15

Asp Lys Met Lys Trp Leu Glu Glu Lys Met Lys Lys Leu Glu Glu Lys
20 25 30

Met Lys Val Met Lys
35

(2) INFORMATION FOR SEQ ID NO:87:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 62 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..62
(D) OTHER INFORMATION: /product= "synthetic oligonucletide" /standard_name= "SM 112"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

GCTCGAAGAA AGATGAAGGC AATGGAAGAC AAAATGAAGT GGCTTGAGGA GAAAATGAAG 60

AA 62

(2) INFORMATION FOR SEQ ID NO:88:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 62 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..62
(D) OTHER INFORMATION: /product= "synthetic oligonucleotide" /standard_name= "SM 113"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

AGCTTCTTCA TTTTCTCCTC AAGCCACTTC ATTTTGTCTT CCATTGCCTT CATCTTTCTT 60

CG 62

(2) INFORMATION FOR SEQ ID NO:89:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

Met Glu Glu Lys Met Lys Lys Leu Lys Glu Glu Met Ala Lys Met Lys
1 5 10 15

Asp Glu Met Trp Lys Leu Lys Glu Glu Met Lys Lys Leu Glu Glu Lys
20 25 30

Met Lys Val Met Lys
35

(2) INFORMATION FOR SEQ ID NO:90:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 63 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..63
(D) OTHER INFORMATION: /product= "synthetic oligonucleotide" /standard_name= "SM 114"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:

GCTCAAGGAG GAAATGGCTA AGATGAAAGA CGAAATCTGG AAACTGAAAG AGGAAATGAA 60

GAA 63

(2) INFORMATION FOR SEQ ID NO:91:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 63 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..63
(D) OTHER INFORMATION: /product= "synthetic oligonucleotide" /standard_name= "SM 115"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:

AGCTTCTTCA TTTCCTCTTT CAGTTTCCAC ATTTCGTCTT TCATCTTAGC CATTTCCTCC 60

TTG 63

(2) INFORMATION FOR SEQ ID NO:92:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 107 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

```
Met Glu Glu Lys Met Lys Lys Leu Lys Glu Glu Met Ala Lys Met Lys
 1               5                  10                  15

Asp Glu Met Trp Lys Leu Lys Glu Glu Met Lys Lys Leu Glu Glu Lys
            20                  25                  30

Met Lys Val Met Glu Glu Lys Met Lys Lys Leu Glu Glu Lys Met Lys
        35                  40                  45

Ala Met Glu Asp Lys Met Lys Trp Leu Glu Glu Lys Met Lys Lys Leu
    50                  55                  60

Glu Glu Lys Met Lys Val Met Glu Glu Lys Met Lys Lys Leu Glu Glu
65                  70                  75                  80

Lys Met Lys Ala Met Glu Asp Lys Met Lys Trp Leu Glu Glu Lys Met
                85                  90                  95

Lys Lys Leu Glu Glu Lys Met Lys Val Met Lys
            100                 105
```

(2) INFORMATION FOR SEQ ID NO:93:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 839 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

```
GGATCCCCCG GGCTGCAGGA ATTCTACGTA CCATATAGTA AGACTTTGTA TATAAGACGT  60

CACCTCTTAC GTGCATGGTT ATATGTGACA TGTGCAGTGA CGTTGTACCA TATAGTAAGA 120

CTTTGTATAT AAGACGTCAC CTCTTACGTG CATGGTTATA TGTGACATGT GCAGTGACGT 180

TAACCGCACC CTCCTTCCCG TCGTTTCCCA TCTCTTCCTC CTTTAGAGCT ACCACTATAT 240

AAATCAGGGC TCATTTTCTC GCTCCTCACA GGCTCATCAG CACCCCGGCA GTGCCACCCC 300

GACTCCCTGC ACCTGCCATG GGTACGCTAG CCCGGGAGAT CTGACAAAGC AGCATTAGTC 360

CGTTGATCGG TGGAAGACCA CTCGTCAGTG TTGAGTTGAA TGTTTGATCA ATAAAATACG 420
```

GCAATGCTGT AAGGGTTGTT TTTTATGCCA TTGATAATAC ACTGTACTGT TCAGTTGTTG 480

AACTCTATTT CTTAGCCATG CCAGTGCTTT TCTTATTTTG AATAACATTA CAGCAAAAAG 540

TTGAAAGACA AAAAAANNNN NCCCCGAACA GAGTGCTTTG GGTCCCAAGC TTCTTTAGAC 600

TGTGTTCGGC GTTCCCCCTA AATTTCTCCC CTATATCTCA CTCACTTGTC ACATCAGCGT 660

TCTCTTTCCC CTATATCTCC ACGCTCTACA GCAGTTCCAC CTATATCAAA CCTCTATACC 720

CCACCACAAC AATATTATAT ACTTTCATCT TCACCTAACT CATGTACCTT CCAATTTTTT 780

TCTACTAATA ATTATTTACG TGCACAGAAA CTTAGGCAAG GGAGAGAGAG AGCGGTACC 839

(2) INFORMATION FOR SEQ ID NO:94:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:

CTAGAAGCCT CGGCAACGTC AGCAACGGCG GAAGAATCCG GTG 43

(2) INFORMATION FOR SEQ ID NO:95:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:95:

CATGCACCGG ATTCTTCCGC CGTTGCTGAC GTTGCCGAGG CTT 43

(2) INFORMATION FOR SEQ ID NO:96:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 55 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:96:

GATCCCATGG CGCCCCTTAA GTCCACCGCC AGCCTCCCCG TCGCCCGCCG CTCCT 55

(2) INFORMATION FOR SEQ ID NO:97:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 55 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:97:

CTAGAGGAGC GGCGGGCGAC GGGGAGGCTG GCGGTGGACT TAAGGGGCGC CATGG 55

(2) INFORMATION FOR SEQ ID NO:98:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 59 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:98:

CATGGCGCCC ACCGTGATGA TGGCCTCGTC GGCCACCGCC GTCGCTCCGT TCCAGGGGC 59

(2) INFORMATION FOR SEQ ID NO:99:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 59 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:99:

TTAAGCCCCT GGAACGGAGC GACGGCGGTG GCCGACGAGG CCATCATCAC GGTGGGCGC 59

(2) INFORMATION FOR SEQ ID NO:100:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:100:

GCGCCCACCG TGATGA 16

(2) INFORMATION FOR SEQ ID NO:101:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:101:

CACCGGATTC TTCCGC 16

(2) INFORMATION FOR SEQ ID NO:102:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 372 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:102:

GTAAGATTGG TAAAGTCCAG CAAGAAAATG AGATAAAAGA GAAGCCTGAA ATGACGAAAA 60

AATCAGGTGT TTTGATTCTT GGTGCTGGAC GTGTGTNTCG CCCAGCTGCT GATTTCCTAG 120

CTTCAGTTAG AACCATTTCG TCACAGCAAT GGTACAAAAC ATATTTCGGA GCAGACTCTG 180

AAGAGAAAAC AGATGTTCAT GTGATTGTCG CGTCTCTGTA TCTTAAGGAT GCCAAAGAGA 240

CGGTTGAAGG TATTTCAGAT GTAGAAGCAG TTCGGCTAGA TGTATCTGAT AGTGAAAGTC 300

TCCTTAAGTA TGTTTCTCAG GTTGATGTTG TCCTAAGTTT ATTACCTGCA AGTTGTCATG 360

CTTGTTGTAG CA 372

(2) INFORMATION FOR SEQ ID NO:103:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 323 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:103:

GGAAGCACAC TGCGACTCTT TTGGAATTCG GGGACATCAA GAATGGACAA ACAACAACCG 60

CTATGGCCAA GACTGTTGGG ATCCCTGCAG CCATTGGAGC TCTGCTGTTA ATTGAAGACA 120

AGATCAAGAC AAGAGGAGTC TTAAGGCCTC TCGAAGCAGA GGTGTATTTG CCAGCTTTGG 180

ATATATTGCA AGCATATGGT ATAAAGCTGA TGGAGAAGGC AGAATGATCA AAGAACTCTG 240

TATATTGTTT CTNTCTATAA CTTGGAGTTG GAGACAAAGC TGAAGGAGNC AGNGCCATTA 300

GACCAGCAAA AAAAGGAGGA GGA 323

(2) INFORMATION FOR SEQ ID NO:104:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 123 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:104:

Lys Ile Gly Lys Val Gln Gln Glu Asn Glu Ile Lys Glu Lys Pro Glu
1 5 10 15

Met Thr Lys Lys Ser Gly Val Leu Ile Leu Gly Ala Gly Arg Val Xaa
20 25 30

Arg Pro Ala Ala Asp Phe Leu Ala Ser Val Arg Thr Ile Ser Ser Gln
35 40 45

Gln Trp Tyr Lys Thr Tyr Phe Gly Ala Asp Ser Glu Glu Lys Thr Asp
50 55 60

Val His Val Ile Val Ala Ser Leu Tyr Leu Lys Asp Ala Lys Glu Thr
65 70 75 80

Val Glu Gly Ile Ser Asp Val Glu Ala Val Arg Leu Asp Val Ser Asp
85 90 95

Ser Glu Ser Leu Leu Lys Tyr Val Ser Gln Val Asp Val Val Leu Ser
100 105 110

Leu Leu Pro Ala Ser Cys His Ala Cys Cys Ser
115 120

(2) INFORMATION FOR SEQ ID NO:105:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 74 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:105:

Lys His Thr Ala Thr Leu Leu Glu Phe Gly Asp Ile Lys Asn Gly Gln
1 5 10 15

Thr Thr Thr Ala Met Ala Lys Thr Val Gly Ile Pro Ala Ala Ile Gly
20 25 30

Ala Leu Leu Leu Ile Glu Asp Lys Ile Lys Thr Arg Gly Val Leu Arg
35 40 45

Pro Leu Glu Ala Glu Val Tyr Leu Pro Ala Leu Asp Ile Leu Gln Ala
50 55 60

Tyr Gly Ile Lys Leu Met Glu Lys Ala Glu
65 70

(2) INFORMATION FOR SEQ ID NO:106:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:106:

ATTCCCCATG GTTTCGCCGA CGAAT 25

(2) INFORMATION FOR SEQ ID NO:107:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:107:

CTCTCGGTAC CTAGTACCTA CTGATCAAC 29

(2) INFORMATION FOR SEQ ID NO:108:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:108:

AGAGAAGCCT GAAATGACGA AAAA 24

(2) INFORMATION FOR SEQ ID NO:109:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:109:

GTCTTGGCCA TAGCGGTTGT TGTT 24

(2) INFORMATION FOR SEQ ID NO:110:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8160 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:110:

TCTAGATGCA CATTCAACTC GAGGTTGTTG CATGATGTTT CATTTACCAA AAAAATCATA 60

GTCAAATTAT GTAAGCAAAT GATATTACAG AAAAGTTTTA CTAGAGAGTT TCAGATTTAC 120

ACATGCACAA CGTTAAAAAA AATAGCAGAA AAAAGAAAGA AGAAAAGTTC TTTATTTGTG 180

AGAAAAATGT ATGAAAAAAA AAGAGATGGG TGTAAAAAGC AAAAGGATAG GACCACTGTT 240

ACTTTGTAGC CTCGTTGAGG AATCTCTTCT CGCATCTCGA CTTTTGTGCC ATTGCAAAGT 300

CAATGCCCAG AACTTGTTCC CAGGCCATCT CCAATTAACT ACGTCTATTT AATTAAACTT 360

TTAAAAGAAA ACCTAATAAA TTAAACAAAA GAAAAGCCGT CAACGAAATC TAAGCTTGCA 420

GCGATATCGA TGAACTGATA CCAAAACAAT GTTCAAGTTT CACTTTCAAA TTGTTTTTTC 480

TTGAAATAGT TTATTGGGTA AGGCCCATAG ATATTTCATA AGAAGAACAC TTGTCGAGGT 540

TGAATCGTAT GTCTGCCCAC CGCGGCCCAT GCATCCTCTG TTGGTAGCAT AATCGTTTTA 600

GGCCATACTA TTGTTCGTAC ACACTGATTT TGAAGTCACC TTTGTGCACT CCTTAATTCC 660

TAAATTGAAG AAGCTTGTTC TCATTCTTCT TTGGGTTACA AATGCCAAGG CAAAAGGAAC 720

TTGGGCCAAA TTAAGACAAC AACTCAAGCC CACTCTCTGC AAATAATACT TGGGAATTTT 780

TACTAAAACG GTGCGTTTCA TCCAAGAATC TATTAATATC CCTAACTTGA AATCATCATA 840

TACGTAACCC AACATATTAA AGAGTTAATA ATGTTAAAAA AAGTCTCAGA AGAGAGAGAC 900

GTAGAGAACA CGGAAAGTGG TAACTGGTAA GCGTCGTCAT CGAGGATATA GTAGCTACGT 960

GAGCAAACGT CTTCACTCAT CTCTGTCTAT TTCTCTTCGA ATACACGTAA TACATTTTCG 1020

ATTGGATTGA TCCTCCCTCG GTCCTATCCA AGTATCCATC CACGTAAACA AGAGCTTGTT 1080

CCTTTCTTGT TTTTTCTTTC TTTAAATAGT AAAAATACTT ATTTCATTTG TTTCGTTTGA 1140

TTTCATTATT ATTGTCTATG GCATTATATA CTATATATAT TATTTCTACA ACATTGGCTG 1200

GCTCACGTTG TTCTCGTGTA TACAACAAAC TTAATTAATG TCTCTCTATT GCATTAGATA 1260

GTTTCGGAGC ATATCCATTA TGTGAAAGCC ACATTAAGTT ATAACTAAAA GTAGTTTTCG 1320

AAAGAGCTTA ATTAAGTTAT GTTCTGTTTC AAATAAAAAT GAACACGAGG GATTTTTTTT 1380

TTTTTTGACA GATCATTATT AACAAAAATG ATTACCTGAA GAAAGGGGAA AATAATTATA 1440

GCTGATTACA GATCATTATT AACAAAAAGA ATTCTTGTCA CATCATTCAT TATAACAAGA 1500

AATATTATAT TATATTAATT TAATCTTTCG CTAACACGCC CACAATATAT TAATCATATA 1560

CGTAATTTAG CTTATAAAAA GGACGGAAAG AGATTATTAC TGCGCCTAAA AAACTCACTA 1620

ATTCCAAAGA AAAAAAAAAG CTTGTATTTT TTCTTGACAA ACCAGCTCAC AGGCATTGCA 1680

TGATCAAACT CATCAGGTAC GTTTTGATTC CTTCTTCCAT AATTTTCCCA TCTTGAGGAA 1740

TGCAAATTTG GAGAGCGCTT TAGCTAAATC ACTGCCTTCA TTTTTTCACT TTGGATTTAA 1800

TAATTTGCAT TCCTCTCTTC CTCTCTGCTC TGTTCTGTTC TGTTCTGTTC TGATTTGAGT 1860

TTTCAATTAA TCGCTCGAGC AAAAGCTATT TCTCAACTCG TTAAATTTCT GTTCCCAGTT 1920

TGTTCGATTT TCAACAGTTT CACATTAAAG TTTGGGTTTT TGATGTTTGG TTGATGAAAC 1980

TCGAAATATG AAATGTTTGT GAATCTATTC CAGGGTGTTT AAAATAAGGG TTTGTTGTTC 2040

ATCTGCAGAG ATTATATGTT TTTACATGAA AGATGAATTC AAATGGCCAT GAGGAGGAGA 2100

AGAAGTTGGG GAATGGAGTT GTGGGGATTC TAGCTGAAAC AGTTAACAAA TGGGAGAGAC 2160

GAACACCATT GACGCCATCG CATTGCGCTC GCCTTTTACA CGGTGGGAAA GACAGAACCG 2220

GCATTTCCCG CATTGTGGTT CAGCCATCTG CTAAGCGTAT CCATCATGAT GCCTTGTATG 2280

AAGATGTTGG GTGTGAAATT TCTGATGATT TGTCTGATTG TGGGCTTATA CTTGGAATCA 2340

AACAACCTGA GGTGTGGGAA TTTGCATTAA AAAGAGTTCC TTTTTTTCTT CTATATATAT 2400

ATCAGTTTAT GAGATTTGAT TCTGTTTGCA GCTAGAAATG ATTCTTCCAG AGAGAGCATA 2460

CGCTTTCTTT TCACATACTC ATAAGGCACA GAAAGAGAAC ATGCCTTTGT TGGATAAAGT 2520

ATTACACTTT TCATTTATCC TTTTAGTCCT ATCTAAGATA CTGAGGAATG TTGACAAAAG 2580

GGGTATCCAA TTGCAGATTC TTTCTGAGAG AGTGACTTTG TGTGATTATG AGCTCATTGT 2640

TGGGGATCAT GGGAAACGAT TATTGGCGTT TGGTAAATAT GCAGGCAGAG CTGGTCTTGT 2700

TGACTTCTTA CACGGACTTG GACAGCGTAA GCTCATGTTA TAATTCTGAT GATCAGGACA 2760

TGTTTCTGTG CAGAACAAGA TGAGATGTAA TTTTCCATGT TTGATGCAGG ATATCTAAGT 2820

CTAGGATACT CAACACCTTT CCTCTCGCTC GGTGCATCGT ATATGTATTC CTCATTGGCT 2880

GCTGCAAAAG CCGCTGTAAT TTCTGTTGGT GAAGAAATTG CAAGCCAGGG ACTGCCATTA 2940

GGAATCTGCC CTCTTGTATT TGTCTTCACC GGAACAGGAA ATGGTATCTT CTTTAGTTCT 3000

ACTGCGAGTT CTTTGAATCC TTCTGCATAT GTTTCATCTC ATTAAAAAAT TTCTCATCCG 3060

CAGTTTCTCT GGGGGCGCAA GAAATTTTCA AGCTTCTTCC TCACACTTTT GTTGAACCAA 3120

GCAAACTTCC TGAACTATTT GTAAAAGTAA GTCACGCTTT GCTTTTTATT TGGTTTCAGA 3180

GTTTTGAAGA TTCTGAAATG TATATTTCTC ACAGGACAAA GGAATTAGTC AAAATGGGAT 3240

TTCAACAAAG CGAGTCTATC AAGTATATGG TTGTATTATT ACCAGCCAAG ACATGGTTGA 3300

ACACAAAGAT CCATCAAAGT CATTCGACAA AGTAACACTT ACCTTCTTAG CTCCTTGGCT 3360

GTGACTTTTG TTCCACTACG CTAAAGTAGA ATACCTATTA ATTCTTCAAG CTTATGATGT 3420

TTAGGCCGAC TATTATGCAC ACCCGGAACA TTACAATCCA GTTTTCCACG AAAAGATATC 3480

GCCATATACG TCTGTTCTTG GTAGATCCTG ATCACTGTTT TACCTTTAAA GCTCAAGAGT 3540

TTACATATAA GCAAATCCTC TGTCCACTCC GTGACTGTGA CCATCTCATT TTGGTTAGTT 3600

CCAGTGTGTA ACCCCTATGA CTTTCTGTGC AGTAAACTGT ATGTACTGGG AGAAGAGGTT 3660

TCCCTGTCTT CTGAGCACAA AACAGCTTCA AGATTTAACA AAAAAAGGAC TCCCACTAGT 3720

AGGCATATGT GATATAACTT GTGACATCGG TGGCTCCATT GAATTTGTTA ACCGAGCTAC 3780

TTTAATCGAT TCCCCTTTCT TCAGGTAATA TATACTTAGG AAGAGCTTTC TTTTGAGTCA 3840

TCTACGTTTA CTATGATGAA ACTCGTCGAG CTAAACACTA TCTCTAGGTT TAATCCCTCG 3900

AACAATTCAT ACTACGATGA CATGGATGGG GATGGCGTAC TATGCATGGC TGTTGACATT 3960

TTACCCACAG AATTTGCAAA AGAGGTATGT ATGAAGGTTA CAGTTATAGT ACTTAAGATT 4020

AAATCTAAAG TTAAAAACCT TGTATTGAGT GGGAGTTCTT GTGTCCTGAA AAAGGCATCC 4080

CAGCATTTTG GAGATATTCT TTCCGGATTT GTCGGTAGTT TGGCTTCAAT GACTGAAATT 4140

TCAGATCTAC CAGCACATCT GAAGAGGGCT TGCATAAGCT ATAGGGGAGA ATTGACATCT 4200

TTGTATGAGT ATATTCCACG TATGAGGAAG TCAAATCCAG AGTATGTTCT GCTTCGAGCG 4260

TTACTTCATC TGAAATATTT AGGCCTCTTC TCTAAACTAT GTTTTCATCT TTACCCACTT 4320

TAACTGCAGA GAGGCACAAG ATAATATTAT CGCCAACGGG GTTTCCAGCC AGAGAACATT 4380

CAACATATTG GTTAGTTTTG ATGAAGAAAG TATATATAAC TAGTTTCCGA ATCATATGAT 4440

TTAAGCTAAT GAATTAAGAA AATATATAGT TCAAGACTTA TGATTCATAT CTCTATCAAC 4500

TTTTTGACCA AAGATTGATA CTTTTTCGAC ATCTGTCACA GCATTTTGTG ATGATTTTGA 4560

TTGAGACAAA TCATTTGTAG GTATCTCTGA GCGGACACCT ATTTGATAAG TTTCTGATAA 4620

ACGAAGCTCT TGATATGATC GAAGCGGCTG GTGGCTCATT TCATTTGGCT AAATGTGAAC 4680

TGGGGCAGAG CGCTGATGCT GAATCGTACT CAGAACTTGA AGTAAGTTTC TTTCTGGATA 4740

AAACCTAATC ATTCACATGG AACAACTGTC AAGAGTTTTT AATGTCACGT TTAGGTTCAA 4800

TGTCCTTTTC ACTAAGTCTC GTAAGTTTTT AAAACAAGTA AACAAACTAC AAGCCAAAAA 4860

CATTCTGGCC CCACATTAAC CTATTCCCAC TTGTTAAAGA ACCCATCTTG CATTATCTTG 4920

GTAGGTTGGT GCGGATGATA AGAGAGTATT GGATCAAATC ATTGATTCAT TAACTCGGTT 4980

AGCTAATCCA AATGAAGATT ATATATCCCC ACATAGAGAA GCAAATAAGA TCTCACTGAA 5040

GATTGGTAAA GTCCAGCAAG AAAATGAGAT AAAAGAGAAG CCTGAAATGA CGAAAAAATC 5100

AGGCGTTTTG ATTCTTGGTG CTGGACGTGT GTGTCGCCCA GCTGCTGATT TCCTAGCTTC 5160

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AGTTTTACTG ATGATCAAAT TTTCCGCAGA CGGTTGAAGG TATTTCAGAT GTAGAAGCAG 5400

TTCGGCTAGA TGTATCTGAT AGTGAAAGTC TCCTTAAGTA TGTTTCTCAG GTATTTTCCT 5460

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TATTTTTTTT TCATTGCACG CAGGTTGATG TTGTCCTAAG TTTATTACCT GCAAGTTGTC 5580

ATGCTGTTGT AGCAAAGACA TGCATTGAGG TAAATTCCTA ACGTTTAATG CGTTTTCCGA 5640

GTGAAGTTAT GAAATTTGCA AATGTTATTC GACATAGAGG TTAAACTTCC TCTGCATAAC 5700

ACATTCTTTC AGTAGTTTCC GGTTCCTAAA TGTCTCTGTT TCTTCTTTCT GATTCACTCA 5760

GCTGAAGAAG CATCTCGTCA CTGCTAGCTA TGTTGATGAT GAAACGTCCA TGTTACATGA 5820

GAAGGCTAAG AGTGCTGGGA TAACGATTCT AGGCGAAATG GGACTGGACC CTGGAATCGG 5880

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GCATTTATCT TCAAAATATT TCCCGGATAA CTGAGAAGGT GATCCTACAA TGAATCTTTC 6000

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TTTAGACATC CTTTCAGAAT TTCGCTTACT CAATTACATC TCGGTATTTT CAGCTGGAAC 6240

CCTGCTGGAG CAATTCGAGC TGGTCAAAAC CCCGCCAAAT ACAAAAGCAA CGGCGACATA 6300

ATACATGTTG ATGGTATGAA AAACAAAATA TGTCTACATG CAGGAGAGGT TGGAGTAGTT 6360

TAGCTTCACT ACACATCATT TTTGTTTAAC CGAGCAATGT AAATCGCAGG GAAGAATCTC 6420

TATGATTCCG CGGCAAGATT CCGAGTACCT AATCTTCCAG CTTTTGCATT GGAGTGTCTT 6480

CCAAATCGTG ACTCCTTGGT TTACGGGGAA CATTATGGCA TCGAGAGCGA AGCAACAACG 6540

ATATTTCGTG GAACACTCAG ATATGAAGGC ATGAATTCCA TAATCACAAC TCACGACTCA 6600

CTTCTCCATA TCTGAAGGCT TAACACTTGT TTTCTTTTGG CTTGTACAGG GTTTAGTATG 6660

ATAATGGCAA CACTTTCGAA ACTTGGATTC TTTGACAGTG AAGCAAATCA AGTACTCTCC 6720

ACTGGAAAGA GGATTACGTT TGGTGCTCTT TTAAGTAACA TTCTAAATAA GGATGCCGAC 6780

AATGAATCAG AGCCCCTAGC GGGAGAAGAA GAGATAAGCA AGAGAATTAT CAAGCTTGGA 6840

CATTCCAAGG AGACTGCAGC CAAAGCTGCC AAAACAATTG TGTAAGCTTC TCCATGAAGA 6900

TATATAATCT GAATGTTGCA GTGTGATTCC AATTCTTCTA CGAAACTCCT AACCCCAATT 6960

CTTTTGTGGT GTCTTAGATT CTTGGGGTTC AACGAAGAGA GGGAGGTTCC ATCACTGTGT 7020

AAAAGCGTAT TTGATGCAAC TTGTTACCTA ATGGAAGAGA AACTAGCTTA TTCCGGAAAT 7080

GAACAGGTCT CTGTTTCATG TGAAAGCATT AGTTTTCTTC TCTCACTTGT ATTTGGTGTT 7140

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GCATCACGAA GTAGAAGTGG AATTCCTTGA AAGCAAACGT ATAGAGAAGC ACACTGCGAC 7260

TCTTTTGGAA TTCGGGGACA TCAAGAATGG GCAAACAACA ACCGCTATGG CCAAGACTGT 7320

TGGGATCCCT GCAGCCATTG GAGCTCTGGT CCTTACTAAG ACTTTGATCA CCACTTTTTC 7380

CTGTCTATAT TTCTCTAAAA TGAAAGTTTT AAGCGTTTGT TTTATGATGT TGTGTGTTGC 7440

AGCTGTTAAT TGAAGACAAG ATCAAGACAA GAGGAGTCTT AAGGCCTTTC GAAGCAGAGG 7500

TGTATTTGCC AGGTAAATTA GAATTCCGCT TCAAAAGGAT GTGTGTTGCA GATAAAGACA 7560

ATGATGTTGA TTTGTTGTGT GTTTGGGATA TGTGGTGTTA TACATACAGC TTTGGATATA 7620

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TGTTTCTCTC TATAACTTGG AGTTGGAGAC AAAGCTGAAG AAGACAGAGA CATTAGACCA 7740

GCAAAAAAAG AAGAAGAAGG AAGAAGATAA GCCTCGATCC TTGGGTGACG AGTATCTATA 7800

TGTTTATATG TACTATATGT TATGTTGTAC AGAAGAAGTC GTGTCCACAA ATATCAATTG 7860

ATGTCAGATG TCTAGTAAGT GATCATGTGT AGCATACAAA CTGGAGTAAT TTAAAAAGTG 7920

AATAAACAAA AATAATTACT AAACGTTATT CCAAGTAGCT TTCCAAGACA GTCACTTGCC 7980

CTTTTCCAAT TTCCCTTGCA ATTAACTAAA TTGCTCTTCA CGATATGATA TTATACCAAA 8040

ATGGTGATAC CTTGGGAATT GTTAATTTGA CTCATTTGAA CAAATCTCAT CTATAAAATC 8100

ATCCCACCTC TCCACCACAT TTGTTCTCAC TACCAATCAA AAAATAATCT AGTCTTAAAC 8160

(2) INFORMATION FOR SEQ ID NO:111:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3194 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:111:

ATGAATTCAA ATGGCCATGA GGAGGAGAAG AAGTTGGGGA ATGGAGTTGT GGGGATTCTA 60

TCTGAAACAG TTAACAAATG GGAGAGACGA ACACCATTGA CGCCATCGCA TTGCGCTCGC 120

CTTTTACACG GTGGGAAAGA CAGAACCGGC ATTTCCCGCA TTGTGGTTCA GCCATCTGCT 180

AAGCGTATCC ATCATGATGC CTTGTATGAA CATGTTGGGT GTGAAATTTC TGATGATTTG 240

TCTGATTGTG GGCTTATACT TGGAATCAAA CAACCTGAGC TAGAAATGAT TCTTCCAGAG 300

AGAGCATACG CTTTCTTTTC ACATACTCAT AAGGCACAGA AAGAGAACAT GCCTTTGTTG 360

GATAAAATTC TTTCTGAGAG AGTGACTTTG TGTGATTATG AGCTCATTGT TGGGGATCAT 420

GGGAAACGAT TATTGGCGTT TGGTAAATAT GCAGGCAGAG CTGGTCTTGT TGACTTCTTA 480

CACGGACTTG GACAGCGATA TCTAAGTCTA GGATACTCAA CACCTTTCCT CTCGCTCGGT 540

GCATCGTATA TGTATTCCTC ATTGGCTGCT GCAAAAGCCG CTGTAATTTC TGTTGGTGAA 600

GAAATTGCAA GCCAGGGACT GCCATTAGGA ATCTGCCCTC TTGTATTTGT CTTCACCGGA 660

ACAGGAAATG TTTCTCTGGG GGCGCAAGAA ATTTTCAAGC TTCTTCCTCA CACTTTTGTT 720

GAACCAAGCA AACTTCCTGA ACTATTTGTA AAAGACAAAG GAATTAGTCA AAATGGGATT 780

TCAACAAAGC GAGTCTATCA AGTATATGGT TGTATTATTA CCAGCCAAGA CATGGTTGAA 840

CACAAAGATC CATCAAAGTC ATTCGACAAA GCCGACTATT ATGCACACCC GGAACATTAC 900

AATCCAGTTT TCCACGAAAA GATATCGCCA TATACGTCTG TTCTTGTAAA CTGTATGTAC 960

TGGGAGAAGA GGTTTCCCTG TCTTCTGAGC ACAAAACAGC TTCAAGATTT AACAAAAAAA 1020

GGACTCCCAC TAGTAGGCAT ATGTGATATA ACTTGTGACA TCGGTGGCTC CATTGAATTT 1080

GTTAACCGAG CTACTTTAAT CGATTCCCCT TTCTTCAGGT TTAATCCCTC GAACAATTCA 1140

TACTACGATG ACATGGATGG GGATGGCGTA CTATGCATGG CTGTTGACAT TTTACCCACA 1200

GAATTTGCAA AAGAGGCATC CCAGCATTTT GGAGATATTC TTTCCGGATT TGTCGGTAGT 1260

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ATCGAAGCGG CTGGTGGCTC ATTTCATTTG GCTAAATGTG AACTGGGGCA GAGCGCTGAT 1560

GCTGAATCGT ACTCAGAACT TGAAGTTGGT GCGGATGATA AGAGAGTATT GGATCAAATC 1620

ATTGATTCAT TAACTCGGTT AGCTAATCCA AATGAAGATT ATATATCCCC ACATAGAGAA 1680

GCAAATAAGA TCTCACTGAA GATTGGTAAA GTCCAGCAAG AAAATGAGAT AAAAGAGAAG 1740

CCTGAAATGA CGAAAAAATC AGGTGTTTTG ATTCTTGGTG CTGGACGTGT GTGTCGCCCA 1800

GCTGCTGATT TCCTAGCTTC AGTTAGAACC ATTTCGTCAC AGCAATGGTA CAAAACATAT 1860

TTCGGAGCAG ACTCTGAAGA GAAAACAGAT GTTCATGTGA TTGTCGCGTC TCTGTATCTT 1920

AAGGATGCCA AAGAGACGGT TGAAGGTATT TCAGATGTAG AAGCAGTTCG GCTAGATGTA 1980

TCTGATAGTG AAAGTCTCCT TAAGTATGTT TCTCAGGTTG ATGTTGTCCT AAGTTTATTA 2040

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ACTGCTAGCT ATGTTGATGA TGAAACGTCC ATGTTACATG AGAAGGCTAA GAGTGCTGGG 2160

ATAACGATTC TAGGCGAAAT GGGACTGGAC CCTGGAATCG ATCACATGAT GGCGATGAAA 2220

ATGATCAACG ATGCTCATAT CAAAAAAGGG AAAGTGAAGT CTTTTACCTC TTATTGTGGA 2280

GGGCTTCCCT CTCCTGCTGC AGCAAATAAT CCATTAGCAT ATAAATTTAG CTGGAACCCT 2340

GCTGGAGCAA TTCGAGCTGG TCAAAACCCC GCCAAATACA AAAGCAACGG CGACATAATA 2400

CATGTTGATG GGAAGAATCT CTATGATTCC GCGGCAAGAT TCCGAGTACC TAATCTTCCA 2460

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ATAATGGCAA CACTTTCGAA ACTTGGATTC TTTGACAGTG AAGCAAATCA AGTACTCTCC 2640

ACTGGAAAGA GGATTACGTT TGGTGCTCTT TTAAGTAACA TTCTAAATAA GGATGCAGAC 2700

AATGAATCAG AGCCCCTAGC GGGAGAAGAA GAGATAAGCA AGAGAATTAT CAAGCTTGGA 2760

CATTCCAAGG AGACTGCAGC CAAAGCTGCC AAAACAATTG TATTCTTGGG GTTCAACGAA 2820

GAGAGGGAGG TTCCATCACT GTGTAAAAGC GTATTTGATG CAACTTGTTA CCTAATGGAA 2880

GAGAAACTAG CTTATTCCGG AAATGAACAG GACATGGTGC TTTTGCATCA CGAAGTAGAA 2940

GTGGAATTCC TTGAAAGCAA ACGTATAGAG AAGCACACTG CGACTCTTTT GGAATTCGGG 3000

GACATCAAGA ATGGACAAAC AACAACCGCT ATGGCCAAGA CTGTTGGGAT CCCTGCAGCC 3060

ATTGGAGCTC TGGTGTTAAT TGAAGACAAG ATCAAGACAA GAGGAGTCTT AAGGCCTCTC 3120

GAAGCAGAGG TGTATTTGCC AGCTTTGGAT ATATTGCAAG CATATGGTAT AAAGCTGATG 3180

GAGAAGGCAG AATGA 3195

(2) INFORMATION FOR SEQ ID NO:112:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1064 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:112:

Met Asn Ser Asn Gly His Glu Glu Glu Lys Lys Leu Gly Asn Gly Val
1 5 10 15

Val Gly Ile Leu Ser Glu Thr Val Asn Lys Trp Glu Arg Arg Thr Pro
20 25 30

Leu Thr Pro Ser His Cys Ala Arg Leu Leu His Gly Gly Lys Asp Arg
35 40 45

Thr Gly Ile Ser Arg Ile Val Val Gln Pro Ser Ala Lys Arg Ile His
50 55 60

His Asp Ala Leu Tyr Glu His Val Gly Cys Glu Ile Ser Asp Asp Leu
65 70 75 80

Ser Asp Cys Gly Leu Ile Leu Gly Ile Lys Gln Pro Glu Leu Glu Met
85 90 95

Ile Leu Pro Glu Arg Ala Tyr Ala Phe Phe Ser His Thr His Lys Ala
100 105 110

Gln Lys Glu Asn Met Pro Leu Leu Asp Lys Ile Leu Ser Glu Arg Val
115 120 125

Thr Leu Cys Asp Tyr Glu Leu Ile Val Gly Asp His Gly Lys Arg Leu
130 135 140

Leu Ala Phe Gly Lys Tyr Ala Gly Arg Ala Gly Leu Val Asp Phe Leu
145 150 155 160

His Gly Leu Gly Gln Arg Tyr Leu Ser Leu Gly Tyr Ser Thr Pro Phe
165 170 175

Leu Ser Leu Gly Ala Ser Tyr Met Tyr Ser Ser Leu Ala Ala Ala Lys
180 185 190

Ala Ala Val Ile Ser Val Gly Glu Glu Ile Ala Ser Gln Gly Leu Pro
195 200 205

Leu Gly Ile Cys Pro Leu Val Phe Val Phe Thr Gly Thr Gly Asn Val
210 215 220

Ser Leu Gly Ala Gln Glu Ile Phe Lys Leu Leu Pro His Thr Phe Val
225 230 235 240

Glu Pro Ser Lys Leu Pro Glu Leu Phe Val Lys Asp Lys Gly Ile Ser
245 250 255

Gln Asn Gly Ile Ser Thr Lys Arg Val Tyr Gln Val Tyr Gly Cys Ile
260 265 270

Ile Thr Ser Gln Asp Met Val Glu His Lys Asp Pro Ser Lys Ser Phe
275 280 285

Asp Lys Ala Asp Tyr Tyr Ala His Pro Glu His Tyr Asn Pro Val Phe
290 295 300

His Glu Lys Ile Ser Pro Tyr Thr Ser Val Leu Val Asn Cys Met Tyr
305 310 315 320

Trp Glu Lys Arg Phe Pro Cys Leu Leu Ser Thr Lys Gln Leu Gln Asp
325 330 335

Leu Thr Lys Lys Gly Leu Pro Leu Val Gly Ile Cys Asp Ile Thr Cys
340 345 350

Asp Ile Gly Gly Ser Ile Glu Phe Val Asn Arg Ala Thr Leu Ile Asp
355 360 365

Ser Pro Phe Phe Arg Phe Asn Pro Ser Asn Asn Ser Tyr Tyr Asp Asp
370 375 380

Met Asp Gly Asp Gly Val Leu Cys Met Ala Val Asp Ile Leu Pro Thr
385 390 395 400

Glu Phe Ala Lys Glu Ala Ser Gln His Phe Gly Asp Ile Leu Ser Gly
405 410 415

Phe Val Gly Ser Leu Ala Ser Met Thr Glu Ile Ser Asp Leu Pro Ala
420 425 430

His Leu Lys Arg Ala Cys Ile Ser Tyr Arg Gly Glu Leu Thr Ser Leu
435 440 445

Tyr Glu Tyr Ile Pro Arg Met Arg Lys Ser Asn Pro Glu Glu Ala Gln
450 455 460

Asp Asn Ile Ile Ala Asn Gly Val Ser Ser Gln Arg Thr Phe Asn Ile
465 470 475 480

Leu Val Ser Leu Ser Gly His Leu Phe Asp Lys Phe Leu Ile Asn Glu
485 490 495

Ala Leu Asp Met Ile Glu Ala Ala Gly Gly Ser Phe His Leu Ala Lys
500 505 510

Cys Glu Leu Gly Gln Ser Ala Asp Ala Glu Ser Tyr Ser Glu Leu Glu
515 520 525

Val Gly Ala Asp Asp Lys Arg Val Leu Asp Gln Ile Ile Asp Ser Leu
530 535 540

Thr Arg Leu Ala Asn Pro Asn Glu Asp Tyr Ile Ser Pro His Arg Glu
545 550 555 560

Ala Asn Lys Ile Ser Leu Lys Ile Gly Lys Val Gln Gln Glu Asn Glu
565 570 575

Ile Lys Glu Lys Pro Glu Met Thr Lys Lys Ser Gly Val Leu Ile Leu
580 585 590

Gly Ala Gly Arg Val Cys Arg Pro Ala Ala Asp Phe Leu Ala Ser Val
595 600 605

Arg Thr Ile Ser Ser Gln Gln Trp Tyr Lys Thr Tyr Phe Gly Ala Asp
610 615 620

Ser Glu Glu Lys Thr Asp Val His Val Ile Val Ala Ser Leu Tyr Leu
625 630 635 640

Lys Asp Ala Lys Glu Thr Val Glu Gly Ile Ser Asp Val Glu Ala Val
645 650 655

Arg Leu Asp Val Ser Asp Ser Glu Ser Leu Leu Lys Tyr Val Ser Gln
660 665 670

Val Asp Val Val Leu Ser Leu Leu Pro Ala Ser Cys His Ala Val Val
675 680 685

Ala Lys Thr Cys Ile Glu Leu Lys Lys His Leu Val Thr Ala Ser Tyr
690 695 700

Val Asp Asp Glu Thr Ser Met Leu His Glu Lys Ala Lys Ser Ala Gly
705 710 715 720

Ile Thr Ile Leu Gly Glu Met Gly Leu Asp Pro Gly Ile Asp His Met
725 730 735

Met Ala Met Lys Met Ile Asn Asp Ala His Ile Lys Lys Gly Lys Val
740 745 750

Lys Ser Phe Thr Ser Tyr Cys Gly Gly Leu Pro Ser Pro Ala Ala Ala
755 760 765

Asn Asn Pro Leu Ala Tyr Lys Phe Ser Trp Asn Pro Ala Gly Ala Ile
770 775 780

Arg Ala Gly Gln Asn Pro Ala Lys Tyr Lys Ser Asn Gly Asp Ile Ile
785 790 795 800

His Val Asp Gly Lys Asn Leu Tyr Asp Ser Ala Ala Arg Phe Arg Val
805 810 815

Pro Asn Leu Pro Ala Phe Ala Leu Glu Cys Phe Pro Asn Arg Asp Ser
820 825 830

Leu Val Tyr Gly Glu His Tyr Gly Ile Glu Ser Glu Ala Thr Thr Ile
835 840 845

Phe Arg Gly Thr Leu Arg Tyr Glu Gly Phe Ser Met Ile Met Ala Thr
850 855 860

Leu Ser Lys Leu Gly Phe Phe Asp Ser Glu Ala Asn Gln Val Leu Ser
865 870 875 880

Thr Gly Lys Arg Ile Thr Phe Gly Ala Leu Leu Ser Asn Ile Leu Asn
885 890 895

Lys Asp Ala Asp Asn Glu Ser Glu Pro Leu Ala Gly Glu Glu Glu Ile
900 905 910

Ser Lys Arg Ile Ile Lys Leu Gly His Ser Lys Glu Thr Ala Ala Lys
915 920 925

Ala Ala Lys Thr Ile Val Phe Leu Gly Phe Asn Glu Glu Arg Glu Val
930 935 940

Pro Ser Leu Cys Lys Ser Val Phe Asp Ala Thr Cys Tyr Leu Met Glu
945 950 955 960

Glu Lys Leu Ala Tyr Ser Gly Asn Glu Gln Asp Met Val Leu Leu His
965 970 975

His Glu Val Glu Val Glu Phe Leu Glu Ser Lys Arg Ile Glu Lys His
980 985 990

Thr Ala Thr Leu Leu Glu Phe Gly Asp Ile Lys Asn Gly Gln Thr Thr
995 1000 1005

Thr Ala Met Ala Lys Thr Val Gly Ile Pro Ala Ala Ile Gly Ala Leu
1010 1015 1020

Val Leu Ile Glu Asp Lys Ile Lys Thr Arg Gly Val Leu Arg Pro Leu
1025 1030 1035 1040

Glu Ala Glu Val Tyr Leu Pro Ala Leu Asp Ile Leu Gln Ala Tyr Gly
1045 1050 1055

Ile Lys Leu Met Glu Lys Ala Glu
1060

(2) INFORMATION FOR SEQ ID NO:113:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:113:

TTYTCICAYA CICAYAARGC ICA 23

(2) INFORMATION FOR SEQ ID NO:114:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:114:

TTYTCCCART ACATRCARTT 20

(2) INFORMATION FOR SEQ ID NO:115:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 619 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:115:

GAAAACATGC CTTTGCTGGA TAAGATTCTA GCTGAGAGGG CATCGTTATA TGACTATGAA 60

TTAATTGTTG GGGACACTGG GAAAAGGTTA CTTGCATTTG GAAAATTCGC TGGTAGGGCT 120

GGAATGATCG ACTTTTTGCG CGGATTAGGA CAGCGGTTTT TAAGTCTTGG ATATTCAACA 180

CCTTTCTTGT CACTTGGATC ATCTTACATG TACCCTTCCC TGGCTGCTGC TAAGGCTGCT 240

GTGATTTCTG TTGGTGAAAA ATTGCGACGC AGGGATTGCC ATTGGGGATT TGTCCCCTGG 300

TTTGTTTATT TACTGGTTCA GGAAATGTTT GTTCTGGTGC ACAGGAGATA TTTAAGCTTC 360

TTCCTCATAC CTTTGTTGAT CCATCTAAAC TACGCGACCT ACATAGAACG GACCCAGATC 420

AACCAAGGCA TGCTTCAAAA AGAGTTTTCC AAGTTTATGG TTGTGTTGTG ACTGCCCAAG 480

ACATGGTTGA ACCCAAAGAT CACGTGATAG TGTTTGACAA AGCAGACTAC TATGCACATC 540

CTGAGCATTA CAATCCCACT TTCCATGAAA AAATAGCACC ATATGCATCT GTTATTGTCA 600

ATTGCATGTA TTGGGAAAA 619

(2) INFORMATION FOR SEQ ID NO:116:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 620 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:116:

GAGAATATGC CACTGTTAGA CAAGATCCTT GAAGAAAGGG TGTCCTTGTT TGATTATGAG 60

CTAATTGTTG GAGATGATGG GAAAAGATCA CTAGCATTTG GGAAATTTGC TGGTAGAGCT 120

GGACTGATAG ATTTCTTACA TGGTCTCGGA CAGCGATATT TGAGCCTTGG ATACTCCACT 180

CCATTTCTCT CTCTGGGACA TCTCATATGT TCCTTCGCTC GCTGCAGCCA AGGCTGCAGT 240

CATTGTCGTT GCAGAAGAGA TAGCAACATT TGGACTTCCA TCCGGAATTT GTCCGATAGT 300

GTTTGTGTTC ACTGGAGTTG GAAACGTCTC TCAGGGTGCG CAGGAGATAT TCAAGTTATT 360

GCCCCATACC TTTGTTGATG CTGAGAAGCT TCCCGAAATT TTTCAGGCCA GGAATCTGTC 420

TAAGCAATCT CAGTCGACCA AGAGAGTATT TCAACTTTAT GGTTGTGTTG TGACCTCTAG 480

AGACATAGTT TCTCACAAGG ATCCCACCAG ACAATTTGAC AAAGGTGACT ATTATGCTCA 540

TCCAGAACAC TACACCCCTG TTTTTCATGA AAGAATTGCT CCATATGCAT CTGTCATCGT 600

AAACTGCATG TATTGGGAAA 620

(2) INFORMATION FOR SEQ ID NO:117:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 206 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:117:

Glu Asn Met Pro Leu Leu Asp Lys Ile Leu Ala Glu Arg Ala Ser Leu
1 5 10 15

Tyr Asp Tyr Glu Leu Ile Val Gly Asp Thr Gly Lys Arg Leu Leu Ala
20 25 30

Phe Gly Lys Phe Ala Gly Arg Ala Gly Met Ile Asp Phe Leu Arg Gly
35 40 45

Leu Gly Gln Arg Phe Leu Ser Leu Gly Tyr Ser Thr Pro Phe Leu Ser
50 55 60

Leu Gly Ser Ser Tyr Met Tyr Pro Ser Leu Ala Ala Ala Lys Ala Ala
65 70 75 80

Val Ile Ser Val Gly Glu Xaa Ile Ala Thr Gln Gly Leu Pro Leu Gly
85 90 95

Ile Cys Pro Leu Val Cys Leu Phe Thr Gly Ser Gly Asn Val Cys Ser
100 105 110

Gly Ala Gln Glu Ile Phe Lys Leu Leu Pro His Thr Phe Val Asp Pro
115 120 125

Ser Lys Leu Arg Asp Leu His Arg Thr Asp Pro Asp Gln Pro Arg His
130 135 140

Ala Ser Lys Arg Val Phe Gln Val Tyr Gly Cys Val Val Thr Ala Gln
145 150 155 160

Asp Met Val Glu Pro Lys Asp His Val Ile Val Phe Asp Lys Ala Asp
165 170 175

Tyr Tyr Ala His Pro Glu His Tyr Asn Pro Thr Phe His Glu Lys Ile
180 185 190

Ala Pro Tyr Ala Ser Val Ile Val Asn Cys Met Tyr Trp Glu
195 200 205

(2) INFORMATION FOR SEQ ID NO:118:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 207 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:118:

Glu Asn Met Pro Leu Leu Asp Lys Ile Leu Glu Glu Arg Val Ser Leu
1 5 10 15

Phe Asp Tyr Glu Leu Ile Val Gly Asp Asp Gly Lys Arg Ser Leu Ala
20 25 30

Phe Gly Lys Phe Ala Gly Arg Ala Gly Leu Ile Asp Phe Leu His Gly
35 40 45

Leu Gly Gln Arg Tyr Leu Ser Leu Gly Tyr Ser Thr Pro Phe Leu Ser
50 55 60

Leu Gly Xaa Ser His Met Xaa Pro Ser Leu Ala Ala Ala Lys Ala Ala
65 70 75 80

Val Ile Val Val Ala Glu Glu Ile Ala Thr Phe Gly Leu Pro Ser Gly
85 90 95

Ile Cys Pro Ile Val Phe Val Phe Thr Gly Val Gly Asn Val Ser Gln
100 105 110

Gly Ala Gln Glu Ile Phe Lys Leu Leu Pro His Thr Phe Val Asp Ala
115 120 125

Glu Lys Leu Pro Glu Ile Phe Gln Ala Arg Asn Leu Ser Lys Gln Ser
130 135 140

Gln Ser Thr Lys Arg Val Phe Gln Leu Tyr Gly Cys Val Val Thr Ser
145 150 155 160

Arg Asp Ile Val Ser His Lys Asp Pro Thr Arg Gln Phe Asp Lys Gly
165 170 175

Asp Tyr Tyr Ala His Pro Glu His Tyr Thr Pro Val Phe His Glu Arg
180 185 190

Ile Ala Pro Tyr Ala Ser Val Ile Val Asn Cys Met Tyr Trp Glu
195 200 205

(2) INFORMATION FOR SEQ ID NO:119:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2582 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Glycine max

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 3..2357

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:119:

TTGAACCCAA AGATCACGTG ATAGTGTTTG ACAAAGCAGA CTACTATTCA CACCCTGAGC 60

ATTACAATCC CACTTTCCAT GAAAAAATAG CACCATATGC ATCTGTTATT GTCAATTGCA 120

TGTATTGGGA GAAAAGATTT CCTCAATTGC CGAGCTATAA GCAGATGCAA GACTTAATGG 180

GCCGGGGGAG CCCCCTTGTT GGAATAGCTG ACATAACGTG TGATATAGGG GGTTCAATTG 240

AGTTTGTTAA CCGCGGTACT TCAATTGATT CACCCTTCTT CAGATATGAT CCCTTAACAA 300

ATTCCTACCA TGATGATATG GAGGGGAATG GAGTGATATG CTTAGCTGTT GACATTCTTC 360

CAACAGAATT TGCAAAGGAG GCTTCCCAAC ATTTTGGAAA CATACTTTCC CAATTTGTTG 420

TAAATTTGGC TTCTGCTACA GACATTACAA AGTTGCCTGC TCACTTAAGG AGAGCTTGCA 480

TAGCCCATAA AGGAGTGCTA ACCTCCTTAT ATGATTATAT CCCACGCATG CGGAGTTCTG 540

ATTCAGAGGA AGTATCAGAA AACGCAGAAA ATTCTCTATC CAACAAAAGG AAGTACAATA 600

TATCGGTGTC TCTGAGTGGT CACTTATTTG ATCAGTTTCT GATAAATGAG GCCTTAGATA 660

TTATTGAAGC TGCAGGAGGC TCCTTCCACT TAGTCAACTG CCATGTGGGT CAGAGCATTG 720

AAGCCGTATC ATTCTCTGAA CTTGAAGTTG GTGCAGATAA CAGGGCTGTT CTGGATCAAA 780

TCATTGATTC TTTAACTGCT ATTGCTAGTC CAACTGAACA TGATAGATTT TCAAATCAAG 840

ATTCAAGTAA AATTTCACTT AAGCTTGGTA AAGTTGAAGA GAATGGCATA GAGAAGGAAT 900

CTGACCCCAG AAAGAAGGCT GCGGTTTTAA TTCTTGGAGC TGGTCGGGTC TGTCAACCAG 960

CTGCTGAAAT GTTATCATCA TTTGGAAGGC CATCATCGAG CCAATGGTAT AAAACATTGT 1020

TGGAAGATGA TTTTGAATGT CAAACTGATG TAGAAGTCAT TGTGGGATCT CTGTACCTGA 1080

AGGATGCAGA GCAGACTGTT GAGGGCATTC CAAATGTAAC CGGAATTCAG CTTGATGTGA 1140

TGGATCGTGC CAATTTGTGT AAGTACATTT CACAGGTTGA CGTTGTTATA AGTTTGCTGC 1200

CCCCAAGTTG TCATATTATT GTAGCAAATG CTTGCATTGA GCTGAAAAAA CATCTTGTCA 1260

CTGCTAGCTA TGTTGATAGC TCCATGTCAA TGCTAAATGA TAAGGCTAAA GATGCTGGCA 1320

TAACAATTCT TGGAGAGATG GGCTTGGACC CAGGAATTGG TCATATGATG GCAATGAAGA 1380

TGATCAACCA AGCACATGTG AGGAAGGGGA AAATAAAGTC TTTCACTTCT TATTGTGGTG 1440

GACTTCCATC TCCTGAAGCT GCTAACAATC CATTAGCATA TAAATTCAGT TGGAATCCTG 1500

CAGGAGCCAT CCGAGCTGGG CGCAATCCTG CCACCTACAA ATGGGGTGGT GAAACTGTAC 1560

ATATTGATGG GGACGATCTT TATGATTCGG CTACAAGACT AAGGCTACCG GACCTTCCTG 1620

CTTTTGCTTT GGAATGTCTC CCAAATCGCA ATTCATTACT TTATGGGGAT TTGTATGGAA 1680

TAACTGAAGC ATCAACCATT TTCCGTGGAA CCCTCCGCTA TGAAGGATTT AGTGAGATCA 1740

TGGGGACACT GTCTAGGATT AGCTTATTTA ACAATGAAGC CCATTCGTTG CTAATGAATG 1800

GACAAAGACC AACTTTCAAA AAATTCTTAT TTGAACTTCT CAAAGTTGTT GGTGATAATC 1860

CAGATGAACT ATTGATAGGA GAGAATGACA TCATGGAGCA AATATTAATA CAAGGGCACT 1920

GCAAAGATCA AAGAACGGCA ATGGAGACAG CAAAAACAAT CATTTTCTTG GGACTTCTTG 1980

ACCAAACTGA AATCCCTGCT TCCTGCAAAA GTGCTTTTGA TGTTGCTTGT TTCCGCATGG 2040

AGGAGAGGTT ATCATACACC AGCACAGAAA AGGATATGGT GCTTTTGCAT CATGAAGTGG 2100

AAATAGAATA CCCAGATAGC CAAATTACAG AGAAGCATAG AGCTACTTTA CTTGAATTTG 2160

GGAAGACTCT TGATGAAAAA ACCACAACTG CCATGGCCCT TACTGTTGGT ATTCCAGCTG 2220

CTGTTGGAGC TTTGCTTTTA TTGACAAACA AAATTCAGAC AAGAGGAGTC TTAAGGCCTA 2280

TCGAACCTGA AGTATACAAT CCAGCACTGG ATATTATAGA AGCTTATGGG ATCAAGTTGA 2340

TAGAGAAGAC CGAGTAATTT GCATYTATGA ATTGATGTAT AGGTGTACAT TAATGTACAC 2400

CATGCAATGT TTGATTTGAA TAAGATAAAA TATAATAATT ACTGCAGTCA TGGAATTGCA 2460

ACTGCCATTC TATGCAACTG TCAGAAATGG ACCACACGGT ACCAGCATAG TTAAAACACT 2520

TAGGCAGATA CCAATTTCAA TTGCAGCAGT ACAATCCAAC CAGTTATGAA GTATGGTTCT 2580

AG 2582

(2) INFORMATION FOR SEQ ID NO:120:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3265 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Zea mays

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 3..3071

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:120:

ATTGTGCCCG CCTTCTGCTA GGAGGAGGCA AGAACGGACC TCGAGTAAAC CGGATTATTG 60

TGCAGCCAAG CACAAGGAGG ATCCATCATG ACGCTCAGTA TGAGGATGCA GGATGCGAGA 120

TTTCAGAAGA CCTGTCAGAA TGCGGCCTTA TCATAGGCAT CAAACAACCC AAGCTGCAGA 180

TGATTCTTTC AGATAGAGCG TACGCTTTCT TTTCACACAC ACACAAAGCC CAAAAAGAGA 240

ATATGCCACT GTTAGACAAG ATCCTTGAAG AAAGGGTGTC CTTGTTTGAT TATGAGCTAA 300

TTGTTGGAGA TGATGGGAAA AGATCACTAG CATTTGGGAA ATTTGCTGGT AGAGCTGGAC 360

TGATAGATTT CTTACATGGT CTCGGACAGC GATATTTGAG CCTTGGATAC TCGACTCCAT 420

TTCTCTCTCT GGGACAATCT CATATGTATC CTTCGCTCGC TGCAGCCAAG GCTGCAGTCA 480

TTGTCGTTGC AGAAGAGATA GCAACATTTG GACTTCCATC CGGAATTTGT CCGATAGTGT 540

TTGTGTTCAC TGGAGTTGGA AACGTCTCTC AGGGTGCGCA GGAGATATTC AAGTTATTGC 600

CCCATACCTT TGTTGATGCT GAGAAGCTTC CCGAAATTTT TCAGGCCAGG AATCTGTCTA 660

AGCAATCTCA GTCGACCAAG AGAGTATTTC AACTTTATGG TTGTGTTGTG ACCTCTAGAG 720

ACATAGTTTC TCACAAGGAT CCCACCAGAC AATTTGACAA AGGTGACTAT TATGCTCATC 780

CAGAACACTA CACCCCTGTT TTTCATGAAA GAATTGCTCC ATATGCATCT GTCATCGTAA 840

ACTGTATGTA TTGGGAGAAG AGGTTTCCAC CATTACTAAA TATGGATCAG TTACAGCAAT 900

TGATGGAGAC TGGTTGTCCT TTAGTCGGCG TTTGTGACAT AACTTGTGAT ATTGGAGGTT 960

CCATTGAATT TATCAACAAG AGTACATCAA TAGAGAGGCC TTTCTTTCGG TATGATCCTT 1020

CTAAGAATTC ATACCATGAT GATATGGAAG GTGCCGGAGT GGTCTGCTTG GCTGTTGACA 1080

TTCTCCCTAC AGAATTCTCT AAAGAGGCCT CCCAACATTT TGGAAACATA CTATCTAGAC 1140

TTGTTGCTAG TTTGGCCTCA GTGAAGCAAC CGGCAGAACT TCCTTCCTAC TTGAGAAGAG 1200

CTTGCATTGC ACATGCTGGC AGATTAACTC CTTTGTATGA ATATATCCCT AGGATGAGAA 1260

ATACTATGAT AGATTTGGCA CCCGCAAAAA CAAATCCATT GCCTGACAAG AAGTATAGCA 1320

CCCTGGTATC TCTCAGTGGG CACCTATTTG ATAAGTTCCT TATAAATGAA GCTTTGGACA 1380

TCATTGAGAC AGCTGGAGGT TCATTTCACT TGGTTAGATG TGAAGTTGGA CAAAGCACGG 1440

ATGATATGTC ATACTCAGAG CTTGAAGTAG GAGCAGATGA TACTGCCACA TTGGATAAAA 1500

TTATTGATTC CTTGACTTCT TTAGCTAATG AACATGGTGG AGATCACGAT GCCGGGCAAG 1560

AAATTGAATT AGCTCTGAAG ATAGGAAAAG TCAATGAGTA TGAAACTGAC GTCACAATTG 1620

ATAAAGGAGG GCCAAAGATT TTAATTCTTG GAGCTGGAAG AGTCTGTCGG CCAGCTGCTG 1680

AGTTTCTGGC ATCTTACCCA GACATATGTA CCTATGGTGT TGATGACCAT GATGCAGATC 1740

AAATTCATGT TATCGTGGCA TCTTTGTATC AAAAAGATGC AGAAGAGACA GTTGATGGTA 1800

TTGAAAATAC AACTGCTACC CAGCTTGATG TTGCTGATAT TGGAAGCCTT TCAGATCTTG 1860

TTTCTCAGGT TGAGGTTGTA ATTAGCTTGC TGCCTGCTAG TTTTCATGCT GCCATTGCAG 1920

GAGTATGCAT AGAGTTGAAG AAGCACATGG TAACGGCAAG CTATGTTGAT GAATCCATGT 1980

CAAACTTGAG CCAAGCTGCC AAAGATGCAG GTGTAACTAT ACTTTGTGAA ATGGGCCTAG 2040

ATCCTGGCAT AGATCACTTG ATGTCAATGA AGATGATTGA TGAAGCTCAT GCACGAAAGG 2100

GAAAAATAAA GGCATTTACA TCTTACTGTG GTGGATTGCC ATCTCCAGCT GCAGCAAACA 2160

ATCCGCTTGC CTATAAATTC AGTTGGAACC CAGCTGGTGC ACTCCGGTCA GGGAAAAATC 2220

CTGCAGTCTA CAAATTTCTT GGTGAGACGA TCCATGTAGA TGGTCATAAC TTGTATGAAT 2280

CAGCAAAGAG GCTCAGACTA CGAGAGCTTC CAGCTTTTGC TCTGGAACAC TTGCCAAATC 2340

GGAATTCCTT GATATATGGT GACCTTTATG GTATCTCCAA AGAAGCATCC ACCATATATA 2400

GGGCTACTYT TCGTTACGAA GGTTTTAGTG AGATTATGGT AACCCTTTCC AAAACTGGGT 2460

TCTTTGATGC TGCAAATCAT CCACTGCTGC AAGATACTAG TCGTCCAACA TATAAGGGTT 2520

TCCTTGATGA ACTACTGAAT AATATCTCCA CAATTAACAC GGACTTAGAT ATTGAAGCTT 2580

CTGGTGGATA CGATGATGAC CTGATTGCCA GACTGTTGAA GCTCGGGTGT TGCAAAAATA 2640

AGGAAATAGC TGTTAAGACA GTCAAAACCA TCAAGTTCTT GGGACTACAT GAAGAGACTC 2700

AAATACCTAA GGGTTGTTCG AGCCCATTTG ATGTGATTTG CCAGCGAATG GAACAGAGGA 2760

TGGCCTATGG CCACAATGAG CAAGACATGG TACTGCTCCA CCACGAAGTC GAGGTGGAAT 2820

ACCCGGACGG GCAACCCGCC GAAAAGCACC AAGCGACGCT ACTGGAGTTC GGGAAGGTTG 2880

AAAATGGCAG GTCCACCACT GCCATGGCGC TGACCGTCGG CATTCCAGCA GCAATAGGGG 2940

CCCTGCTATT GCTAAAGAAT AAGGTCCAGA CGAAAGGAGT GATCAGGCCT CTGCAACCGG 3000

AAATCTACGT TCCAGCATTG GAGATCTTGG AGTCGTCGGG CATCAAGCTG GTTGAGAAAG 3060

TGGAGACTTG AAAGTTCCCT GATACACAGA TAAAGATAGT ATGATATAGC AGGGCACATG 3120

TATCTTTTGT ATTAACTCCG TTCTGGAATA TATATTTGTG AACTAAAATG TGACAAATAA 3180

AAAGAACGGG TGGAGTATAT TGTAAGAGAC GGCAAAGAAA CCTCTGTATA TATGACCTGT 3240

CGATATCAAA TAATGCCGAT CAGTT
3265

(2) INFORMATION FOR SEQ ID NO:121:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 784 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Glycine max

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:121:

Glu Pro Lys Asp His Val Ile Val Phe Asp Lys Ala Asp Tyr Tyr Ser
1 5 10 15

His Pro Glu His Tyr Asn Pro Thr Phe His Glu Lys Ile Ala Pro Tyr
20 25 30

Ala Ser Val Ile Val Asn Cys Met Tyr Trp Glu Lys Arg Phe Pro Gln
35 40 45

Leu Pro Ser Tyr Lys Gln Met Gln Asp Leu Met Gly Arg Gly Ser Pro
50 55 60

Leu Val Gly Ile Ala Asp Ile Thr Cys Asp Ile Gly Gly Ser Ile Glu
65 70 75 80

Phe Val Asn Arg Gly Thr Ser Ile Asp Ser Pro Phe Phe Arg Tyr Asp
85 90 95

Pro Leu Thr Asn Ser Tyr His Asp Asp Met Glu Gly Asn Gly Val Ile
100 105 110

Cys Leu Ala Val Asp Ile Leu Pro Thr Glu Phe Ala Lys Glu Ala Ser
115 120 125

Gln His Phe Gly Asn Ile Leu Ser Gln Phe Val Val Asn Leu Ala Ser
130 135 140

Ala Thr Asp Ile Thr Lys Leu Pro Ala His Leu Arg Arg Ala Cys Ile
145 150 155 160

Ala His Lys Gly Val Leu Thr Ser Leu Tyr Asp Tyr Ile Pro Arg Met
165 170 175

Arg Ser Ser Asp Ser Glu Glu Val Ser Glu Asn Ala Glu Asn Ser Leu
180 185 190

Ser Asn Lys Arg Lys Tyr Asn Ile Ser Val Ser Leu Ser Gly His Leu
195 200 205

Phe Asp Gln Phe Leu Ile Asn Glu Ala Leu Asp Ile Ile Glu Ala Ala
210 215 220

Gly Gly Ser Phe His Leu Val Asn Cys His Val Gly Gln Ser Ile Glu
225 230 235 240

Ala Val Ser Phe Ser Glu Leu Glu Val Gly Ala Asp Asn Arg Ala Val
245 250 255

Leu Asp Gln Ile Ile Asp Ser Leu Thr Ala Ile Ala Ser Pro Thr Glu
260 265 270

His Asp Arg Phe Ser Asn Gln Asp Ser Ser Lys Ile Ser Leu Lys Leu
275 280 285

Gly Lys Val Glu Glu Asn Gly Ile Glu Lys Glu Ser Asp Pro Arg Lys
290 295 300

Lys Ala Ala Val Leu Ile Leu Gly Ala Gly Arg Val Cys Gln Pro Ala
305 310 315 320

Ala Glu Met Leu Ser Ser Phe Gly Arg Pro Ser Ser Ser Gln Trp Tyr
325 330 335

Lys Thr Leu Leu Glu Asp Asp Phe Glu Cys Gln Thr Asp Val Glu Val
340 345 350

Ile Val Gly Ser Leu Tyr Leu Lys Asp Ala Glu Gln Thr Val Glu Gly
355 360 365

Ile Pro Asn Val Thr Gly Ile Gln Leu Asp Val Met Asp Arg Ala Asn
370 375 380

Leu Cys Lys Tyr Ile Ser Gln Val Asp Val Val Ile Ser Leu Leu Pro
385 390 395 400

Pro Ser Cys His Ile Ile Val Ala Asn Ala Cys Ile Glu Leu Lys Lys
405 410 415

His Leu Val Thr Ala Ser Tyr Val Asp Ser Ser Met Ser Met Leu Asn
420 425 430

Asp Lys Ala Lys Asp Ala Gly Ile Thr Ile Leu Gly Glu Met Gly Leu
435 440 445

Asp Pro Gly Ile Gly His Met Met Ala Met Lys Met Ile Asn Gln Ala
450 455 460

His Val Arg Lys Gly Lys Ile Lys Ser Phe Thr Ser Tyr Cys Gly Gly
465 470 475 480

Leu Pro Ser Pro Glu Ala Ala Asn Asn Pro Leu Ala Tyr Lys Phe Ser
485 490 495

Trp Asn Pro Ala Gly Ala Ile Arg Ala Gly Arg Asn Pro Ala Thr Tyr
500 505 510

Lys Trp Gly Gly Glu Thr Val His Ile Asp Gly Asp Asp Leu Tyr Asp
        515   520   525

Ser Ala Thr Arg Leu Arg Leu Pro Asp Leu Pro Ala Phe Ala Leu Glu
    530   535   540

Cys Leu Pro Asn Arg Asn Ser Leu Leu Tyr Gly Asp Leu Tyr Gly Ile
545   550   555   560

Thr Glu Ala Ser Thr Ile Phe Arg Gly Thr Leu Arg Tyr Glu Gly Phe
                565   570   575

Ser Glu Ile Met Gly Thr Leu Ser Arg Ile Ser Leu Phe Asn Asn Glu
            580   585   590

Ala His Ser Leu Leu Met Asn Gly Gln Arg Pro Thr Phe Lys Lys Phe
        595   600   605

Leu Phe Glu Leu Leu Lys Val Val Gly Asp Asn Pro Asp Glu Leu Leu
    610   615   620

Ile Gly Glu Asn Asp Ile Met Glu Gln Ile Leu Ile Gln Gly His Cys
625   630   635   640

Lys Asp Gln Arg Thr Ala Met Glu Thr Ala Lys Thr Ile Ile Phe Leu
                645   650   655

Gly Leu Leu Asp Gln Thr Glu Ile Pro Ala Ser Cys Lys Ser Ala Phe
            660   665   670

Asp Val Ala Cys Phe Arg Met Glu Glu Arg Leu Ser Tyr Thr Ser Thr
        675   680   685

Glu Lys Asp Met Val Leu Leu His His Glu Val Glu Ile Glu Tyr Pro
    690   695   700

Asp Ser Gln Ile Thr Glu Lys His Arg Ala Thr Leu Leu Glu Phe Gly
705   710   715   720

Lys Thr Leu Asp Glu Lys Thr Thr Thr Ala Met Ala Leu Thr Val Gly
                725   730   735

Ile Pro Ala Ala Val Gly Ala Leu Leu Leu Leu Thr Asn Lys Ile Gln
            740   745   750

Thr Arg Gly Val Leu Arg Pro Ile Glu Pro Glu Val Tyr Asn Pro Ala
        755   760   765

Leu Asp Ile Ile Glu Ala Tyr Gly Ile Lys Leu Ile Glu Lys Thr Glu
    770   775   780

(2) INFORMATION FOR SEQ ID NO:122:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1022 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Zea mays

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:122:

```
Cys Ala Arg Leu Leu Leu Gly Gly Gly Lys Asn Gly Pro Arg Val Asn
1               5                  10                  15

Arg Ile Ile Val Gln Pro Ser Thr Arg Arg Ile His His Asp Ala Gln
            20                  25                  30

Tyr Glu Asp Ala Gly Cys Glu Ile Ser Glu Asp Leu Ser Glu Cys Gly
        35                  40                  45

Leu Ile Ile Gly Ile Lys Gln Pro Lys Leu Gln Met Ile Leu Ser Asp
    50                  55                  60

Arg Ala Tyr Ala Phe Phe Ser His Thr His Lys Ala Gln Lys Glu Asn
65                  70                  75                  80

Met Pro Leu Leu Asp Lys Ile Leu Glu Glu Arg Val Ser Leu Phe Asp
                85                  90                  95

Tyr Glu Leu Ile Val Gly Asp Asp Gly Lys Arg Ser Leu Ala Phe Gly
            100                 105                 110

Lys Phe Ala Gly Arg Ala Gly Leu Ile Asp Phe Leu His Gly Leu Gly
        115                 120                 125

Gln Arg Tyr Leu Ser Leu Gly Tyr Ser Thr Pro Phe Leu Ser Leu Gly
    130                 135                 140

Gln Ser His Met Tyr Pro Ser Leu Ala Ala Ala Lys Ala Ala Val Ile
145                 150                 155                 160

Val Val Ala Glu Glu Ile Ala Thr Phe Gly Leu Pro Ser Gly Ile Cys
                165                 170                 175

Pro Ile Val Phe Val Phe Thr Gly Val Gly Asn Val Ser Gln Gly Ala
            180                 185                 190

Gln Glu Ile Phe Lys Leu Leu Pro His Thr Phe Val Asp Ala Glu Lys
        195                 200                 205

Leu Pro Glu Ile Phe Gln Ala Arg Asn Leu Ser Lys Gln Ser Gln Ser
    210                 215                 220

Thr Lys Arg Val Phe Gln Leu Tyr Gly Cys Val Val Thr Ser Arg Asp
225                 230                 235                 240

Ile Val Ser His Lys Asp Pro Thr Arg Gln Phe Asp Lys Gly Asp Tyr
                245                 250                 255

Tyr Ala His Pro Glu His Tyr Thr Pro Val Phe His Glu Arg Ile Ala
            260                 265                 270

Pro Tyr Ala Ser Val Ile Val Asn Cys Met Tyr Trp Glu Lys Arg Phe
        275                 280                 285

Pro Pro Leu Leu Asn Met Asp Gln Leu Gln Gln Leu Met Glu Thr Gly
    290                 295                 300

Cys Pro Leu Val Gly Val Cys Asp Ile Thr Cys Asp Ile Gly Gly Ser
305                 310                 315                 320
```

Ile Glu Phe Ile Asn Lys Ser Thr Ser Ile Glu Arg Pro Phe Phe Arg
325 330 335

Tyr Asp Pro Ser Lys Asn Ser Tyr His Asp Asp Met Glu Gly Ala Gly
340 345 350

Val Val Cys Leu Ala Val Asp Ile Leu Pro Thr Glu Phe Ser Lys Glu
355 360 365

Ala Ser Gln His Phe Gly Asn Ile Leu Ser Arg Leu Val Ala Ser Leu
370 375 380

Ala Ser Val Lys Gln Pro Ala Glu Leu Pro Ser Tyr Leu Arg Arg Ala
385 390 395 400

Cys Ile Ala His Ala Gly Arg Leu Thr Pro Leu Tyr Glu Tyr Ile Pro
405 410 415

Arg Met Arg Asn Thr Met Ile Asp Leu Ala Pro Ala Lys Thr Asn Pro
420 425 430

Leu Pro Asp Lys Lys Tyr Ser Thr Leu Val Ser Leu Ser Gly His Leu
435 440 445

Phe Asp Lys Phe Leu Ile Asn Glu Ala Leu Asp Ile Ile Glu Thr Ala
450 455 460

Gly Gly Ser Phe His Leu Val Arg Cys Glu Val Gly Gln Ser Thr Asp
465 470 475 480

Asp Met Ser Tyr Ser Glu Leu Glu Val Gly Ala Asp Asp Thr Ala Thr
485 490 495

Leu Asp Lys Ile Ile Asp Ser Leu Thr Ser Leu Ala Asn Glu His Gly
500 505 510

Gly Asp His Asp Ala Gly Gln Glu Ile Glu Leu Ala Leu Lys Ile Gly
515 520 525

Lys Val Asn Glu Tyr Glu Thr Asp Val Thr Ile Asp Lys Gly Gly Pro
530 535 540

Lys Ile Leu Ile Leu Gly Ala Gly Arg Val Cys Arg Pro Ala Ala Glu
545 550 555 560

Phe Leu Ala Ser Tyr Pro Asp Ile Cys Thr Tyr Gly Val Asp Asp His
565 570 575

Asp Ala Asp Gln Ile His Val Ile Val Ala Ser Leu Tyr Gln Lys Asp
580 585 590

Ala Glu Glu Thr Val Asp Gly Ile Glu Asn Thr Thr Ala Thr Gln Leu
595 600 605

Asp Val Ala Asp Ile Gly Ser Leu Ser Asp Leu Val Ser Gln Val Glu
610 615 620

Val Val Ile Ser Leu Leu Pro Ala Ser Phe His Ala Ala Ile Ala Gly
625 630 635 640

Val Cys Ile Glu Leu Lys Lys His Met Val Thr Ala Ser Tyr Val Asp
645 650 655

Glu Ser Met Ser Asn Leu Ser Gln Ala Ala Lys Asp Ala Gly Val Thr
660 665 670

Ile Leu Cys Glu Met Gly Leu Asp Pro Gly Ile Asp His Leu Met Ser
675 680 685

Met Lys Met Ile Asp Glu Ala His Ala Arg Lys Gly Lys Ile Lys Ala
690 695 700

Phe Thr Ser Tyr Cys Gly Gly Leu Pro Ser Pro Ala Ala Ala Asn Asn
705 710 715 720

Pro Leu Ala Tyr Lys Phe Ser Trp Asn Pro Ala Gly Ala Leu Arg Ser
725 730 735

Gly Lys Asn Pro Ala Val Tyr Lys Phe Leu Gly Glu Thr Ile His Val
740 745 750

Asp Gly His Asn Leu Tyr Glu Ser Ala Lys Arg Leu Arg Leu Arg Glu
755 760 765

Leu Pro Ala Phe Ala Leu Glu His Leu Pro Asn Arg Asn Ser Leu Ile
770 775 780

Tyr Gly Asp Leu Tyr Gly Ile Ser Lys Glu Ala Ser Thr Ile Tyr Arg
785 790 795 800

Ala Thr Xaa Arg Tyr Glu Gly Phe Ser Glu Ile Met Val Thr Leu Ser
805 810 815

Lys Thr Gly Phe Phe Asp Ala Ala Asn His Pro Leu Leu Gln Asp Thr
820 825 830

Ser Arg Pro Thr Tyr Lys Gly Phe Leu Asp Glu Leu Leu Asn Asn Ile
835 840 845

Ser Thr Ile Asn Thr Asp Leu Asp Ile Glu Ala Ser Gly Gly Tyr Asp
850 855 860

Asp Asp Leu Ile Ala Arg Leu Leu Lys Leu Gly Cys Cys Lys Asn Lys
865 870 875 880

Glu Ile Ala Val Lys Thr Val Lys Thr Ile Lys Phe Leu Gly Leu His
885 890 895

Glu Glu Thr Gln Ile Pro Lys Gly Cys Ser Ser Pro Phe Asp Val Ile
900 905 910

Cys Gln Arg Met Glu Gln Arg Met Ala Tyr Gly His Asn Glu Gln Asp
915 920 925

Met Val Leu Leu His His Glu Val Glu Val Glu Tyr Pro Asp Gly Gln
930 935 940

Pro Ala Glu Lys His Gln Ala Thr Leu Leu Glu Phe Gly Lys Val Glu
945 950 955 960

Asn Gly Arg Ser Thr Thr Ala Met Ala Leu Thr Val Gly Ile Pro Ala
965 970 975

Ala Ile Gly Ala Leu Leu Leu Leu Lys Asn Lys Val Gln Thr Lys Gly
980 985 990

Val Ile Arg Pro Leu Gln Pro Glu Ile Tyr Val Pro Ala Leu Glu Ile
995 1000 1005

Leu Glu Ser Ser Gly Ile Lys Leu Val Glu Lys Val Glu Thr
1010 1015 1020

(2) INFORMATION FOR SEQ ID NO:123:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1908 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Zea mays

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 3..1908

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:123:

ATTGTGCCCG CCTTCTGCTA GGAGGAGGCA AGAACGGACC TCGAGTAAAC CGGATTATTG 60

TGCAGCCAAG CACAAGGAGG ATCCATCATG ACGCTCAGTA TGAGGATGCA GGATGCGAGA 120

TTTCAGAAGA CCTGTCAGAA TGCGGCCTTA TCATAGGCAT CAAACAACCC AAGCTGCAGA 180

TGATTCTTTC AGATAGAGCG TACGCTTTCT TTTCACACAC ACACAAAGCC CAAAAAGAGA 240

ATATGCCACT GTTAGACAAG ATCCTTGAAG AAAGGGTGTC CTTGTTTGAT TATGAGCTAA 300

TTGTTGGAGA TGATGGGAAA AGATCACTAG CATTTGGGAA ATTTGCTGGT AGAGCTGGAC 360

TGATAGATTT CTTACATGGT CTCGGACAGC GATATTTGAG CCTTGGATAC TCGACTCCAT 420

TTCTCTCTCT GGGACAATCT CATATGTATC CTTCGCTCGC TGCAGCCAAG GCTGCAGTCA 480

TTGTCGTTGC AGAAGAGATA GCAACATTTG GACTTCCATC CGGAATTTGT CCGATAGTGT 540

TTGTGTTCAC TGGAGTTGGA AACGTCTCTC AGGGTGCGCA GGAGATATTC AAGTTATTGC 600

CCCATACCTT TGTTGATGCT GAGAAGCTTC CCGAAATTTT TCAGGCCAGG AATCTGTCTA 660

AGCAATCTCA GTCGACCAAG AGAGTATTTC AACTTTATGG TTGTGTTGTG ACCTCTAGAG 720

ACATAGTTTC TCACAAGGAT CCCACCAGAC AATTTGACAA AGGTGACTAT TATGCTCATC 780

CAGAACACTA CACCCCTGTT TTTCATGAAA GAATTGCTCC ATATGCATCT GTCATCGTAA 840

ACTGTATGTA TTGGGAGAAG AGGTTTCCAC CATTACTAAA TATGGATCAG TTACAGCAAT 900

TGATGGAGAC TGGTTGTCCT TTAGTCGGCG TTTGTGACAT AACTTGTGAT ATTGGAGGTT 960

CCATTGAATT TATCAACAAG AGTACATCAA TAGAGAGGCC TTTCTTTCGG TATGATCCTT 1020

CTAAGAATTC ATACCATGAT GATATGGAAG GTGCCGGAGT GGTCTGCTTG GCTGTTGACA 1080

TTCTCCCTAC AGAATTCTCT AAAGAGGCCT CCCAACATTT TGGAAACATA CTATCTAGAC 1140

TTGTTGCTAG TTTGGCCTCA GTGAAGCAAC CGGCAGAACT TCCTTCCTAC TTGAGAAGAG 1200

CTTGCATTGC ACATGCTGGC AGATTAACTC CTTTGTATGA ATATATCCCT AGGATGAGAA 1260

ATACTATGAT AGATTTGGCA CCCGCAAAAA CAAATCCATT GCCTGACAAG AAGTATAGCA 1320

CCCTGGTATC TCTCAGTGGG CACCTATTTG ATAAGTTCCT TATAAATGAA GCTTTGGACA 1380

TCATTGAGAC AGCTGGAGGT TCATTTCACT TGGTTAGATG TGAAGTTGGA CAAAGCACGG 1440

ATGATATGTC ATACTCAGAG CTTGAAGTAG GAGCAGATGA TACTGCCACA TTGGATAAAA 1500

TTATTGATTC CTTGACTTCT TTAGCTAATG AACATGGTGG AGATCACGAT GCCGGGCAAG 1560

AAATTGAATT AGCTCTGAAG ATAGGAAAAG TCAATGAGTA TGAAACTGAC GTCACAATTG 1620

ATAAAGGAGG GCCAAAGATT TTAATTCTTG GAGCTGGAAG AGTCTGTCGG CCAGCTGCTG 1680

AGTTTCTGGC ATCTTACCCA GACATATGTA CCTATGGTGT TGATGACCAT GATGCAGATC 1740

AAATTCATGT TATCGTGGCA TCTTTGTATC AAAAAGATGC AGAAGAGACA GTTGATGGTA 1800

TTGAAAATAC AACTGCTACC CAGCTTGATG TTGCTGATAT TGGAAGCCTT TCAGATCTTG 1860

TTTCTCAGGT TGAGGTTGTA ATTAGCTTGC TGCCTGCTAG TTTTCATG 1908

(2) INFORMATION FOR SEQ ID NO:124:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 640 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Zea mays

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:124:

Cys Ala Arg Leu Leu Leu Gly Gly Gly Lys Asn Gly Pro Arg Val Asn
1 5 10 15

Arg Ile Ile Val Gln Pro Ser Thr Arg Arg Ile His His Asp Ala Gln
20 25 30

Tyr Glu Asp Ala Gly Cys Glu Ile Ser Glu Asp Leu Ser Glu Cys Gly
35 40 45

Leu Ile Ile Gly Ile Lys Gln Pro Lys Leu Gln Met Ile Leu Ser Asp
50 55 60

Arg Ala Tyr Ala Phe Phe Ser His Thr His Lys Ala Gln Lys Glu Asn
65 70 75 80

Met Pro Leu Leu Asp Lys Ile Leu Glu Glu Arg Val Ser Leu Phe Asp
85 90 95

Tyr Glu Leu Ile Val Gly Asp Asp Gly Lys Arg Ser Leu Ala Phe Gly
100 105 110

Lys Phe Ala Gly Arg Ala Gly Leu Ile Asp Phe Leu His Gly Leu Gly
115 120 125

Gln Arg Tyr Leu Ser Leu Gly Tyr Ser Thr Pro Phe Leu Ser Leu Gly
130 135 140

Gln Ser His Met Tyr Pro Ser Leu Ala Ala Ala Lys Ala Ala Val Ile
145 150 155 160

Val Val Ala Glu Glu Ile Ala Thr Phe Gly Leu Pro Ser Gly Ile Cys
165 170 175

Pro Ile Val Phe Val Phe Thr Gly Val Gly Asn Val Ser Gln Gly Ala
180 185 190

Gln Glu Ile Phe Lys Leu Leu Pro His Thr Phe Val Asp Ala Glu Lys
195 200 205

Leu Pro Glu Ile Phe Gln Ala Arg Asn Leu Ser Lys Gln Ser Gln Ser
210 215 220

Thr Lys Arg Val Phe Gln Leu Tyr Gly Cys Val Val Thr Ser Arg Asp
225 230 235 240

Ile Val Ser His Lys Asp Pro Thr Arg Gln Phe Asp Lys Gly Asp Tyr
245 250 255

Tyr Ala His Pro Glu His Tyr Thr Pro Val Phe His Glu Arg Ile Ala
260 265 270

Pro Tyr Ala Ser Val Ile Val Asn Cys Met Tyr Trp Glu Lys Arg Phe
275 280 285

Pro Pro Leu Leu Asn Met Asp Gln Leu Gln Gln Leu Met Glu Thr Gly
290 295 300

Cys Pro Leu Val Gly Val Cys Asp Ile Thr Cys Asp Ile Gly Gly Ser
305 310 315 320

Ile Glu Phe Ile Asn Lys Ser Thr Ser Ile Glu Arg Pro Phe Phe Arg
325 330 335

Tyr Asp Pro Ser Lys Asn Ser Tyr His Asp Asp Met Glu Gly Ala Gly
340 345 350

Val Val Cys Leu Ala Val Asp Ile Leu Pro Thr Glu Phe Ser Lys Glu
355 360 365

Ala Ser Gln His Phe Gly Asn Ile Leu Ser Arg Leu Val Ala Ser Leu
370 375 380

Ala Ser Val Lys Gln Pro Ala Glu Leu Pro Ser Tyr Leu Arg Arg Ala
385 390 395 400

Cys Ile Ala His Ala Gly Arg Leu Thr Pro Leu Tyr Glu Tyr Ile Pro
405 410 415

Arg Met Arg Asn Thr Met Ile Asp Leu Ala Pro Ala Lys Thr Asn Pro
420 425 430

Leu Pro Asp Lys Lys Tyr Ser Thr Leu Val Ser Leu Ser Gly His Leu
435 440 445

Phe Asp Lys Phe Leu Ile Asn Glu Ala Leu Asp Ile Ile Glu Thr Ala
450 455 460

Gly Gly Ser Phe His Leu Val Arg Cys Glu Val Gly Gln Ser Thr Asp
465 470 475 480

Asp Met Ser Tyr Ser Glu Leu Glu Val Gly Ala Asp Asp Thr Ala Thr
485 490 495

Leu Asp Lys Ile Ile Asp Ser Leu Thr Ser Leu Ala Asn Glu His Gly
500 505 510

Gly Asp His Asp Ala Gly Gln Glu Ile Glu Leu Ala Leu Lys Ile Gly
515 520 525

Lys Val Asn Glu Tyr Glu Thr Asp Val Thr Ile Asp Lys Gly Gly Pro
530 535 540

Lys Ile Leu Ile Leu Gly Ala Gly Arg Val Cys Arg Pro Ala Ala Glu
545 550 555 560

Phe Leu Ala Ser Tyr Pro Asp Ile Cys Thr Tyr Gly Val Asp Asp His
565 570 575

Asp Ala Asp Gln Ile His Val Ile Val Ala Ser Leu Tyr Gln Lys Asp
580 585 590

Ala Glu Glu Thr Val Asp Gly Ile Glu Asn Thr Thr Ala Thr Gln Leu
595 600 605

Asp Val Ala Asp Ile Gly Ser Leu Ser Asp Leu Val Ser Gln Val Glu
610 615 620

Val Val Ile Ser Leu Leu Pro Ala Ser Phe His Ala Ala Ile Ala Gly
625 630 635 640

(2) INFORMATION FOR SEQ ID NO:125:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 720 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Oryza sativa

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2..720

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 215
(D) OTHER INFORMATION: /label= unknown

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 678
(D) OTHER INFORMATION: /label= unknown

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:125:

GTTTAAACAT CTTTCCAATC TTGTTTCTCA GGTTGAAGTA GTAGTTAGCT TGCTGCCTGC 60

CAGTTTTCAT GCTGCCATAG CAAGAGTATG CATAGAGATG AAGAAGCACT TGGTCACTGC 120

AAGCTATGTT GATGAGTCCA TGTCAAAGTT GGAACAATCT GCAGAAGGTG CTGGTGTAAC 180

TATTCTCTGT GAAATGGGCC TGGATCCTGG CATANATCAT ATGATGTCAA TGAAGATGAT 240

TGACGAAGCA CATTCACGGA AGGGGAAAAT AAAGTCATTT ACATCCTTTT GTGGAGGACT 300

TCCATCTCCA GCTTCTGCAA ACAATCCACT TGCTTATAAG TTCAGTTGGA GTCCAGCTGG 360

TGCCATCCGT GCAGGGAGAA ACCCTGCTGT CTACAAATTT CATGGAGAAA TCATCCATGT 420

AGATGGTGAT AAATTGTATG AATCCGCAAA GAGGCTCAGA TTACMAGAAC TTCCAGCTTT 480

TGCACTGGAA CACTTGCCAA ACCGGAATTC CTTGATGTAT GGAGACCTGT ATGGGATCTC 540

CAAAGAAGCA TCTACTGTGT ACAGGGCTAC TCTTCGTTAT GAAGGATTTA ATGAGATAAT 600

GGCAACCTTC GCGAAAATTG GGTTTTTTGA TGCTGCAAGT CATCCACTGT TGCAACAAAC 660

TACTCGCCCT ACATACANGG ATTTCCTGTT GAACCCTCAA TGCTTGTACA TCTCCAAAAC 720

(2) INFORMATION FOR SEQ ID NO:126:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 239 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Oryza sativa

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:126:

Phe Lys His Leu Ser Asn Leu Val Ser Gln Val Glu Val Val Val Ser
1 5 10 15

Leu Leu Pro Ala Ser Phe His Ala Ala Ile Ala Arg Val Cys Ile Glu
20 25 30

Met Lys Lys His Leu Val Thr Ala Ser Tyr Val Asp Glu Ser Met Ser
35 40 45

Lys Leu Glu Gln Ser Ala Glu Gly Ala Gly Val Thr Ile Leu Cys Glu
50 55 60

Met Gly Leu Asp Pro Gly Ile Xaa His Met Met Ser Met Lys Met Ile
65 70 75 80

Asp Glu Ala His Ser Arg Lys Gly Lys Ile Lys Ser Phe Thr Ser Phe
85 90 95

Cys Gly Gly Leu Pro Ser Pro Ala Ser Ala Asn Asn Pro Leu Ala Tyr
100 105 110

Lys Phe Ser Trp Ser Pro Ala Gly Ala Ile Arg Ala Gly Arg Asn Pro
115 120 125

Ala Val Tyr Lys Phe His Gly Glu Ile Ile His Val Asp Gly Asp Lys
130 135 140

Leu Tyr Glu Ser Ala Lys Arg Leu Arg Leu Xaa Glu Leu Pro Ala Phe
145 150 155 160

Ala Leu Glu His Leu Pro Asn Arg Asn Ser Leu Met Tyr Gly Asp Leu
165 170 175

Tyr Gly Ile Ser Lys Glu Ala Ser Thr Val Tyr Arg Ala Thr Leu Arg
180 185 190

Tyr Glu Gly Phe Asn Glu Ile Met Ala Thr Phe Ala Lys Ile Gly Phe
195 200 205

Phe Asp Ala Ala Ser His Pro Leu Leu Gln Gln Thr Thr Arg Pro Thr
210 215 220

Tyr Xaa Asp Phe Leu Leu Asn Pro Gln Cys Leu Tyr Ile Ser Lys
225 230 235

(2) INFORMATION FOR SEQ ID NO:127:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 308 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Oryza sativa

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..129

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:127:

CTGCTGTTGC TCCAGAACAA GATCCAAAAG AAAGGAGTGA TCAGGCCTCT GGAACCTGAA 60

ATTTACATTC CAGCGTTGGA GATCTTGGAG TCATCGGGTA TCAAGCTGGC GGAGAGAGTG 120

GAGACCTGAG AATCGGACCC AATATGTATA ATGTAGCATG GTGGTAGCTT CTCTATATAT 180

ATGCTTCAGT GAATAATTGA TTTGCCGTTG TGTGGTAATT AAGCAATGCC CGCTAATAAA 240

TTGTACCGTA GAAGTCCTTC TATGTACATC CGTATCAAAA AATAAAAAAA GCATCGATTA 300

GCTTGAAT 308

(2) INFORMATION FOR SEQ ID NO:128:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 42 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Oryza sativa

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:128:

```
Leu Leu Leu Leu Gln Asn Lys Ile Gln Lys Lys Gly Val Ile Arg Pro
1               5                  10                  15

Leu Glu Pro Glu Ile Tyr Ile Pro Ala Leu Glu Ile Leu Glu Ser Ser
            20                  25                  30

Gly Ile Lys Leu Ala Glu Arg Val Glu Thr
        35                  40
```

(2) INFORMATION FOR SEQ ID NO:129:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 429 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Triticum aestivum

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..252

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 172
(D) OTHER INFORMATION: /label= unknown

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 186
(D) OTHER INFORMATION: /label= unknown

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 331
(D) OTHER INFORMATION: /label= unknown

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:129:

TACCCCGACG GGGACCCCAC CGAGAAGCAC CAAGCGACGC TGCTGGAGTT CGGAAAGACC 60

GAGAACGGCA GGCCCACCAC CGCCATGGCC CTCACCGTTG GGGTACCGGC AGCGATAGGA 120

GCCCTGCTCT TGCTCCAGAA CAAGGTCCAG AGGAAAGGGG TGATCCGGCC TNTGGAACCG 180

GAGATNTACA TCCCTGCGCT GGAGATCTTG GAAGCGTCGG GCATCAAGCT GATCGAGAGA 240

GTGGAGACCT GAGGATGTCA GGATGGGATG AGAATCTATC GAGTATATAT GCTGCAGCAA 300

CAGAGGCAGT GAGTAAATAA AATGATGATT NTCGCCGTTG TAAGTAAAAT GAGTGGACTG 360

TATGTATGTA TGTGACTATC TATTGTACTA CATATATACC AAATCTGTCG CCGGTTGATT 420

CTGTTGGTG 429

(2) INFORMATION FOR SEQ ID NO:130:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 83 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Triticum aestivum

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:130:

Tyr Pro Asp Gly Asp Pro Thr Glu Lys His Gln Ala Thr Leu Leu Glu
1 5 10 15

Phe Gly Lys Thr Glu Asn Gly Arg Pro Thr Thr Ala Met Ala Leu Thr
20 25 30

Val Gly Val Pro Ala Ala Ile Gly Ala Leu Leu Leu Leu Gln Asn Lys
35 40 45

Val Gln Arg Lys Gly Val Ile Arg Pro Xaa Glu Pro Glu Xaa Tyr Ile
50 55 60

Pro Ala Leu Glu Ile Leu Glu Ala Ser Gly Ile Lys Leu Ile Glu Arg
65 70 75 80

Val Glu Thr

(2) INFORMATION FOR SEQ ID NO:131:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1449 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:131:

ATGACGAAAA AATCAGGTGT TTTGATTCTT GGTGCTGGAC GTGTGTGTCG CCCAGCTGCT 60

GATTTCCTAG CTTCAGTTAG AACCATTTCG TCACAGCAAT GGTACAAAAC ATATTTCGGA 120

GCAGACTCTG AAGAGAAAAC AGATGTTCAT GTGATTGTCG CGTCTCTGTA TCTTAAGGAT 180

GCCAAAGAGA CGGTTGAAGG TATTTCAGAT GTAGAAGCAG TTCGGCTAGA TGTATCTGAT 240

AGTGAAAGTC TCCTTAAGTA TGTTTCTCAG GTTGATGTTG TCCTAAGTTT ATTACCTGCA 300

AGTTGTCATG CTGTTGTAGC AAAGACATGC ATTGAGCTGA AGAAGCATCT CGTCACTGCT 360

AGCTATGTTG ATGATGAAAC GTCCATGTTA CATGAGAAGG CTAAGAGTGC TGGGATAACG 420

ATTCTAGGCG AAATGGGACT GGACCCTGGA ATCGATCACA TGATGGCGAT GAAAATGATC 480

AACGATGCTC ATATCAAAAA AGGGAAAGTG AAGTCTTTTA CCTCTTATTG TGGAGGGCTT 540

CCCTCTCCTG CTGCAGCAAA TAATCCATTA GCATATAAAT TTAGCTGGAA CCCTGCTGGA 600

GCAATTCGAG CTGGTCAAAA CCCCGCCAAA TACAAAAGCA ACGGCGACAT AATACATGTT 660

GATGGGAAGA ATCTCTATGA TTCCGCGGCA AGATTCCGAG TACCTAATCT TCCAGCTTTT 720

GCATTGGAGT GTTTTCCAAA TCGTGACTCC TTGGTTTACG GGGAACATTA TGGCATCGAG 780

AGCGAAGCAA CAACGATATT TCGTGGAACA CTCAGATATG AAGGGTTTAG TATGATAATG 840

GCAACACTTT CGAAACTTGG ATTCTTTGAC AGTGAAGCAA ATCAAGTACT CTCCACTGGA 900

AAGAGGATTA CGTTTGGTGC TCTTTTAAGT AACATTCTAA ATAAGGATGC AGACAATGAA 960

TCAGAGCCCC TAGCGGGAGA AGAAGAGATA AGCAAGAGAA TTATCAAGCT TGGACATTCC 1020

AAGGAGACTG CAGCCAAAGC TGCCAAAACA ATTGTATTCT TGGGGTTCAA CGAAGAGAGG 1080

GAGGTTCCAT CACTGTGTAA AAGCGTATTT GATGCAACTT GTTACCTAAT GGAAGAGAAA 1140

CTAGCTTATT CCGGAAATGA ACAGGACATG GTGCTTTTGC ATCACGAAGT AGAAGTGGAA 1200

TTCCTTGAAA GCAAACGTAT AGAGAAGCAC ACTGCGACTC TTTTGGAATT CGGGGACATC 1260

AAGAATGGAC AAACAACAAC CGCTATGGCC AAGACTGTTG GGATCCCTGC AGCCATTGGA 1320

GCTCTGGTGT TAATTGAAGA CAAGATCAAG ACAAGAGGAG TCTTAAGGCC TCTCGAAGCA 1380

GAGGTGTATT TGCCAGCTTT GGATATATTG CAAGCATATG GTATAAAGCT GATGGAGAAG 1440

GCAGAATGA 1449

(2) INFORMATION FOR SEQ ID NO:132:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 482 amino acids

(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:132:

```
Met Thr Lys Lys Ser Gly Val Leu Ile Leu Gly Ala Gly Arg Val Cys
1               5                  10                  15

Arg Pro Ala Ala Asp Phe Leu Ala Ser Val Arg Thr Ile Ser Ser Gln
            20                  25                  30

Gln Trp Tyr Lys Thr Tyr Phe Gly Ala Asp Ser Glu Glu Lys Thr Asp
        35                  40                  45

Val His Val Ile Val Ala Ser Leu Tyr Leu Lys Asp Ala Lys Glu Thr
    50                  55                  60

Val Glu Gly Ile Ser Asp Val Glu Ala Val Arg Leu Asp Val Ser Asp
65                  70                  75                  80

Ser Glu Ser Leu Leu Lys Tyr Val Ser Gln Val Asp Val Val Leu Ser
                85                  90                  95

Leu Leu Pro Ala Ser Cys His Ala Val Val Ala Lys Thr Cys Ile Glu
           100                 105                 110

Leu Lys Lys His Leu Val Thr Ala Ser Tyr Val Asp Asp Glu Thr Ser
        115                 120                 125

Met Leu His Glu Lys Ala Lys Ser Ala Gly Ile Thr Ile Leu Gly Glu
    130                 135                 140

Met Gly Leu Asp Pro Gly Ile Asp His Met Met Ala Met Lys Met Ile
145                 150                 155                 160

Asn Asp Ala His Ile Lys Lys Gly Lys Val Lys Ser Phe Thr Ser Tyr
                165                 170                 175

Cys Gly Gly Leu Pro Ser Pro Ala Ala Ala Asn Asn Pro Leu Ala Tyr
            180                 185                 190

Lys Phe Ser Trp Asn Pro Ala Gly Ala Ile Arg Ala Gly Gln Asn Pro
        195                 200                 205

Ala Lys Tyr Lys Ser Asn Gly Asp Ile Ile His Val Asp Gly Lys Asn
    210                 215                 220

Leu Tyr Asp Ser Ala Ala Arg Phe Arg Val Pro Asn Leu Pro Ala Phe
225                 230                 235                 240

Ala Leu Glu Cys Phe Pro Asn Arg Asp Ser Leu Val Tyr Gly Glu His
                245                 250                 255

Tyr Gly Ile Glu Ser Glu Ala Thr Thr Ile Phe Arg Gly Thr Leu Arg
            260                 265                 270
```

Tyr Glu Gly Phe Ser Met Ile Met Ala Thr Leu Ser Lys Leu Gly Phe
275 280 285

Phe Asp Ser Glu Ala Asn Gln Val Leu Ser Thr Gly Lys Arg Ile Thr
290 295 300

Phe Gly Ala Leu Leu Ser Asn Ile Leu Asn Lys Asp Ala Asp Asn Glu
305 310 315 320

Ser Glu Pro Leu Ala Gly Glu Glu Glu Ile Ser Lys Arg Ile Ile Lys
325 330 335

Leu Gly His Ser Lys Glu Thr Ala Ala Lys Ala Ala Lys Thr Ile Val
340 345 350

Phe Leu Gly Phe Asn Glu Glu Arg Glu Val Pro Ser Leu Cys Lys Ser
355 360 365

Val Phe Asp Ala Thr Cys Tyr Leu Met Glu Glu Lys Leu Ala Tyr Ser
370 375 380

Gly Asn Glu Gln Asp Met Val Leu Leu His His Glu Val Glu Val Glu
385 390 395 400

Phe Leu Glu Ser Lys Arg Ile Glu Lys His Thr Ala Thr Leu Leu Glu
405 410 415

Phe Gly Asp Ile Lys Asn Gly Gln Thr Thr Thr Ala Met Ala Lys Thr
420 425 430

Val Gly Ile Pro Ala Ala Ile Gly Ala Leu Val Leu Ile Glu Asp Lys
435 440 445

Ile Lys Thr Arg Gly Val Leu Arg Pro Leu Glu Ala Glu Val Tyr Leu
450 455 460

Pro Ala Leu Asp Ile Leu Gln Ala Tyr Gly Ile Lys Leu Met Glu Lys
465 470 475 480

Ala Glu

What is claimed is:

1. An isolated nucleic acid fragment comprising a nucleic acid sequence encoding all or part of lysine ketoglutarate reductase.

2. The nucleic acid fragment of Claim 1 wherein the nucleic acid sequence encodes a polypeptide essentially similar to the polypeptide described by SEQ ID NO:104, SEQ ID NO:105, SEQ ID NO:112, SEQ ID NO:117, SEQ ID NO:118, SEQ ID NO:121, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128, SEQ ID NO:130 or SEQ ID NO:132.

3. The nucleic acid fragment of Claim 1 comprising a nucleic acid sequence wherein the nucleic acid sequence is essentially similar to that of SEQ ID NO:110, SEQ ID NO:111, SEQ ID NO:115, SEQ ID NO:116, SEQ ID NO:119, SEQ ID NO:120, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129 or SEQ ID NO:131.

4. The nucleic acid fragment of Claim 1 comprising a nucleic acid sequence of SEQ ID NO:110, SEQ ID NO:111, SEQ ID NO:115, SEQ ID NO:116, SEQ ID NO:119, SEQ ID NO:120, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129 or SEQ ID NO:131.

5. The nucleic acid fragment of Claim 1 wherein the nucleic acid sequence encodes a polypeptide as set forth in SEQ ID NO:104, SEQ ID NO:105, SEQ ID NO:112, SEQ ID NO:117, SEQ ID NO:118, SEQ ID NO:121, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128, SEQ ID NO:130 or SEQ ID NO:132.

6. A chimeric gene comprising the isolated nucleic acid fragment of Claim 1 encoding lysine ketoglutarate reductase or a subfragment thereof, operably linked to suitable seed-specific regulatory sequences wherein said chimeric gene reduces lysine ketoglutarate reductase activity in seeds of plants transformed with the chimeric gene.

7. The chimeric gene according to Claim 6 wherein the isolated nucleic acid fragment comprises a nucleic acid sequence or subsequence thereof essentially similar to that of SEQ ID NO:110, SEQ ID NO:111, SEQ ID NO:115, SEQ ID NO:116, SEQ ID NO:119, SEQ ID NO:120, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129 or SEQ ID NO:131.

8. A plant cell wherein lysine ketoglutarate reductase activity is reduced due to a mutation in a gene encoding lysine ketoglutarate reductase.

9. A plant cell transformed with the chimeric gene of Claim 6 or 7 wherein said transformed plant cell has reduced lysine ketoglutarate reductase activity.

10. A plant seed wherein lysine ketoglutarate reductase activity is reduced due to a mutation in a gene encoding lysine ketoglutarate reductase.

11. A plant seed transformed with the chimeric gene of Claim 6 or 7 wherein said transformed plant seed has reduced lysine ketoglutarate reductase activity.

12. The plant cell according to Claim 9 wherein said plant cell is selected from the group of plants consisting of *Arabidopsis*, corn, soybean, rapeseed, wheat and rice.

13. The plant seed according to Claim 11 wherein said plant cell is selected from the group of plants consisting of *Arabidopsis*, corn, soybean, rapeseed, wheat and rice.

14. A method for reducing lysine ketoglutarate reductase activity in a plant seed which comprises:

(a) transforming plant cells with the chimeric gene of claim 6 or 7;

(b) regenerating fertile mature plants from the transformed plant cells obtained from step (a) under conditions suitable to obtain seeds;

(c) screening progeny seed of step (b) for reduced lysine ketoglutarate reductase activity; and

(d) selecting those lines whose seeds contain reduced lysine ketoglutarate reductase activity.

15. Seed obtained from the plant of Claim 14.

16. A nucleic acid fragment comprising

(a) a first chimeric gene of Claim 6 or 7 and

(b) a second chimeric gene wherein a nucleic acid fragment encoding dihydrodipicolinic acid synthase which is substantially insensitive to inhibition by lysine is operably linked to a plant chloroplast transit sequence and to a plant seed-specific regulatory sequence.

17. A plant comprising in its genome a first chimeric gene of Claim 6 or 7 wherein said gene reduces lysine ketoglutarate reductase activity in seeds of transformed plants and a second chimeric gene wherein a nucleic acid fragment encoding dihydrodipicolinic acid synthase which is substantially insensitive to inhibition by lysine is operably linked to a plant chloroplast transit sequence and to a plant seed-specific regulatory sequence.

18. A plant comprising in its genome the nucleic acid fragment of Claim 16.

19. Seed obtained from the plant of Claim 17 comprising in its genome the first and second chimeric genes.

20. Seed obtained from the plant of Claim 18 comprising in its genome the nucleic fragment of Claim 16.

TITLE

CHIMERIC GENES AND METHODS FOR INCREASING THE LYSINE CONTENT OF THE SEEDS OF PLANTS

ABSTRACT

Chimeric genes are disclosed. One chimeric gene encodes a plant lysine ketoglutarate reductase and a second chimeric gene encodes lysine-insensitive dihydrodipicolinic acid synthase (DHDPS) which is operably linked to a plant chloroplast transit sequence, all operably linked to plant seed-specific regulatory sequences. Methods for their use to produce increased levels of lysine in the seeds of transformed plants are provided.

LMC/bjm

Rev. 06/95

Docket Number
BB-1037-F

DECLARATION and POWER OF ATTORNEY

As a below-named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Chimeric Genes And Methods For Increasing The Lysine And Threonine Content Of The Seeds Of Plants

the specification of which is attached hereto unless the following box is checked:

☐ was filed on _____ as U.S. Application No. _____ or PCT International Application No. _____ and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is known to me to be material to patentability as defined in 37 CFR § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Application No.	Country	Filing Date	Priority Claimed (Yes/No)

I hereby claim the benefit under 35 U.S.C. § 119(e) of any United States Provisional Application(s) listed below.

U.S. Provisional Application No.	U.S. Filing Date

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or § 365(c) of any PCT International Application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application or PCT International Application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is known to me to be material to patentability as defined in 37 CFR § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

Application No.	Filing Date	Status (patented, pending or abandoned)
07/855,414	03/19/92	Abandoned
PCT/US93/02480	03/18/93	Pending
08/178,212	01/06/94	Abandoned
08/474,633	06/07/95	Pending
08/824,627	03/27/97	Pending

POWER OF ATTORNEY: I hereby appoint the following attorney(s) and/or agent(s) the power to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

Name:	Registration No.:
LYNNE M. CHRISTENBURY	30,971
BARBARA C. SIEGELL	30,684

Send correspondence and direct telephone calls to:		Tel. No.
LYNNE M. CHRISTENBURY	**E. I. du Pont de Nemours and Company Legal - Patents Wilmington, DE 19898, U.S.A.**	(302) 992-5481

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

INVENTOR(S)

Full Name of Inventor	Last Name **FALCO**	First Name **SAVERIO**	Middle Name **CARL**	
	Signature (please sign full name):		Date:	
Residence & Citizenship	City **ARDEN**	State or Foreign Country **DELAWARE**	Country of Citizenship **U.S.A.**	
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Full Name of Inventor	Last Name **MCDEVITT III**	First Name **RAYMOND**	Middle Name **ERVIN**	
	Signature (please sign full name):		Date:	
Residence & Citizenship	City **ARDEN**	State or Foreign Country **DELAWARE**	Country of Citizenship **U.S.A.**	
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■ Additional Inventors are being named on separately numbered sheets attached hereto.

DECLARATION AND POWER OF ATTORNEY - Page 2

Docket No.: **BB-1037-F**

Full Name of Inventor	Last Name **EPELBAUM**	First Name **SABINE**	Middle Name **URSULA**	
[redacted]	Signature (please sign full name):		Date:	
Residence & Citizenship	City **WILMINGTON**	State or Foreign Country **DELAWARE**	Country of Citizenship **ISRAEL**	
Post Office Address	Post Office Address **115C PRESIDENTIAL DRIVE**	City **WILMINGTON**	State or Country **DELAWARE**	Zip Code **19807**

FIG.1

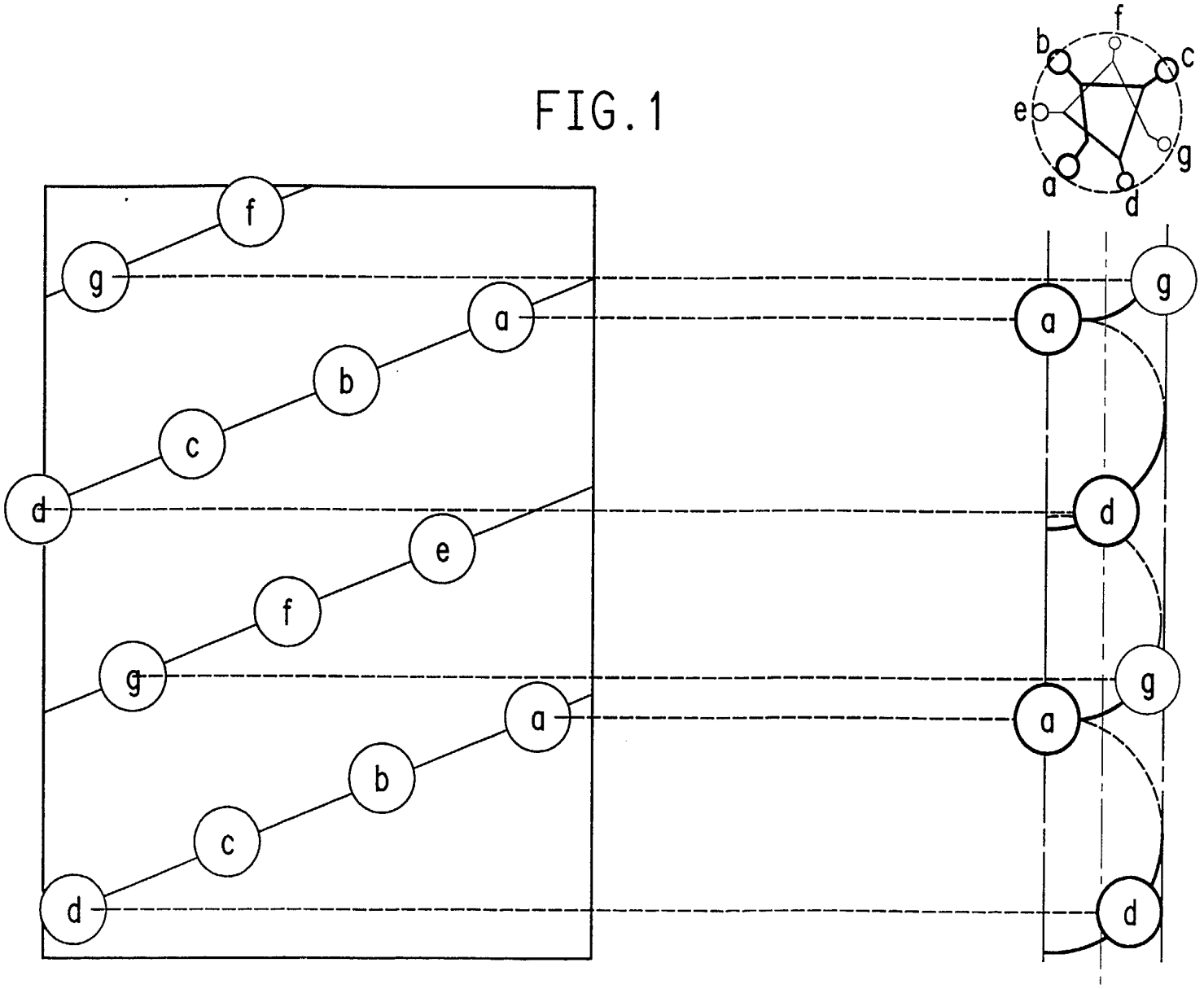


FIG.2A

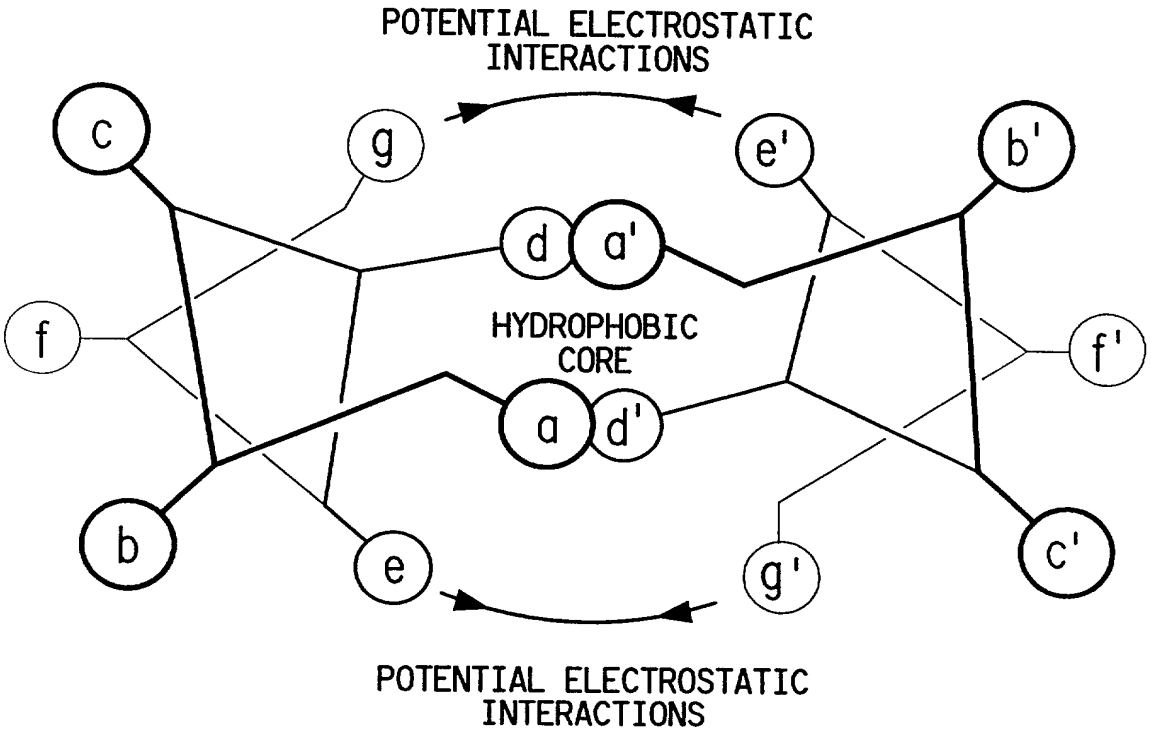


FIG.2B

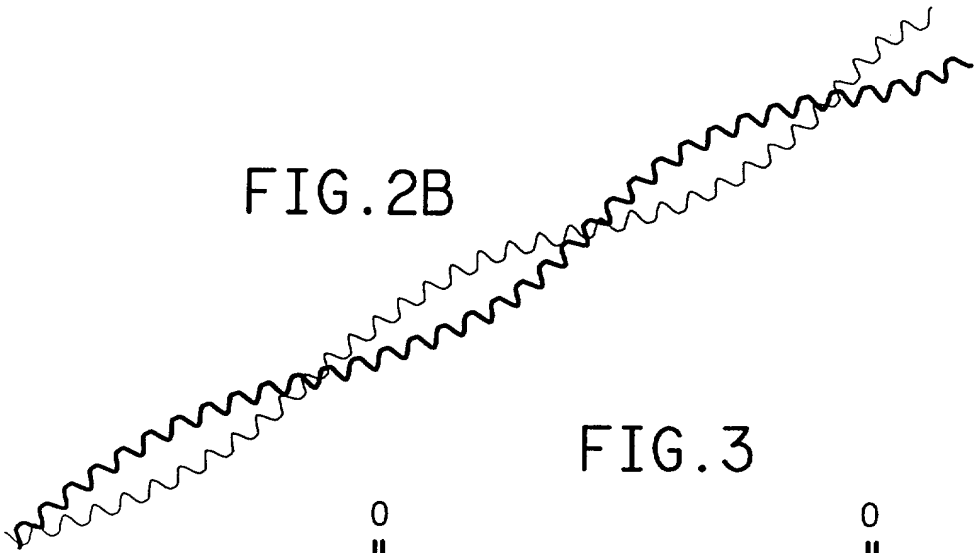


FIG.3

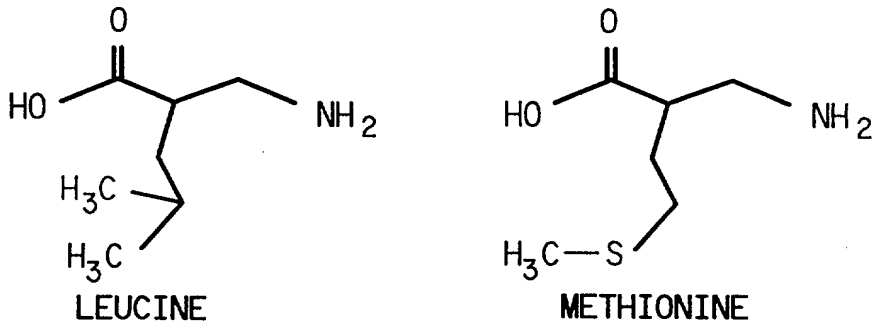


FIG.4a

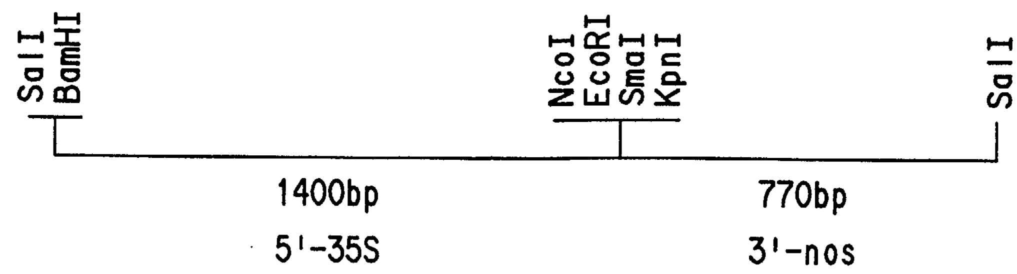


FIG.4b

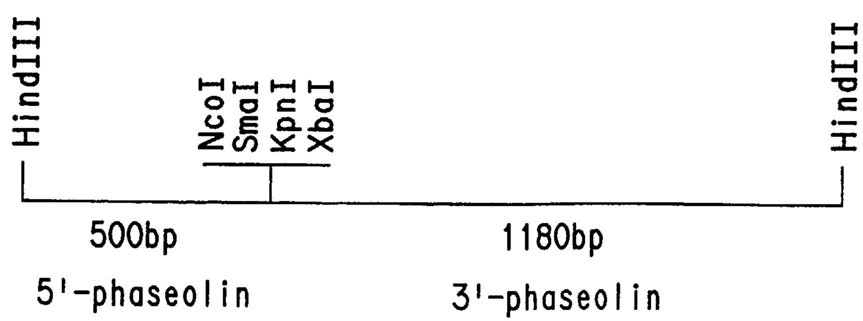
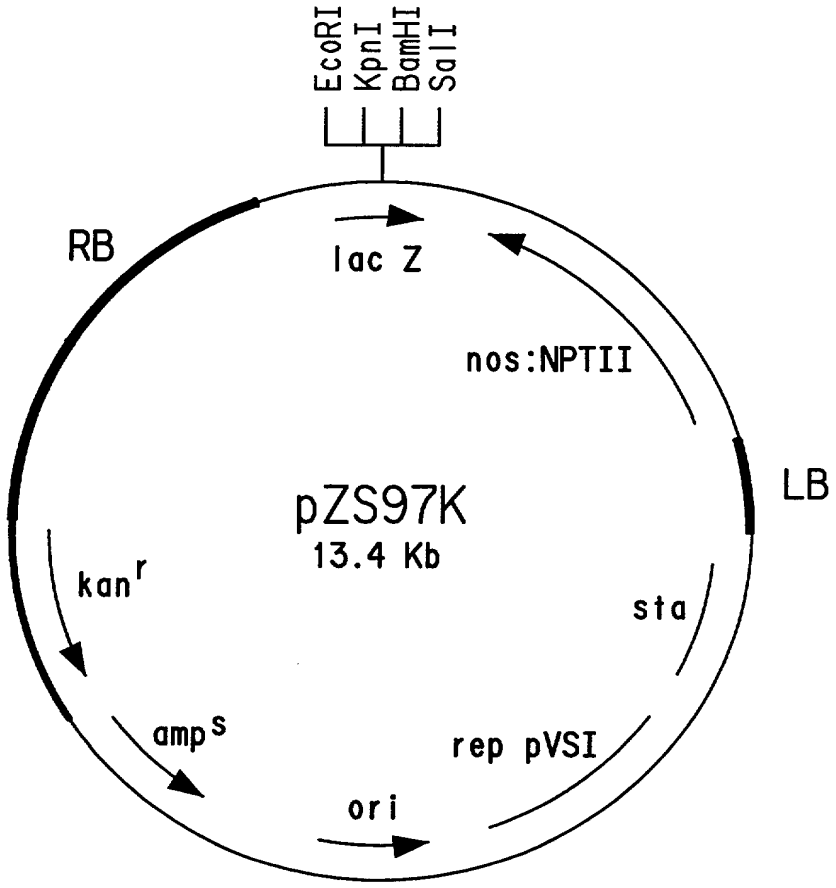


FIG.5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lac Z
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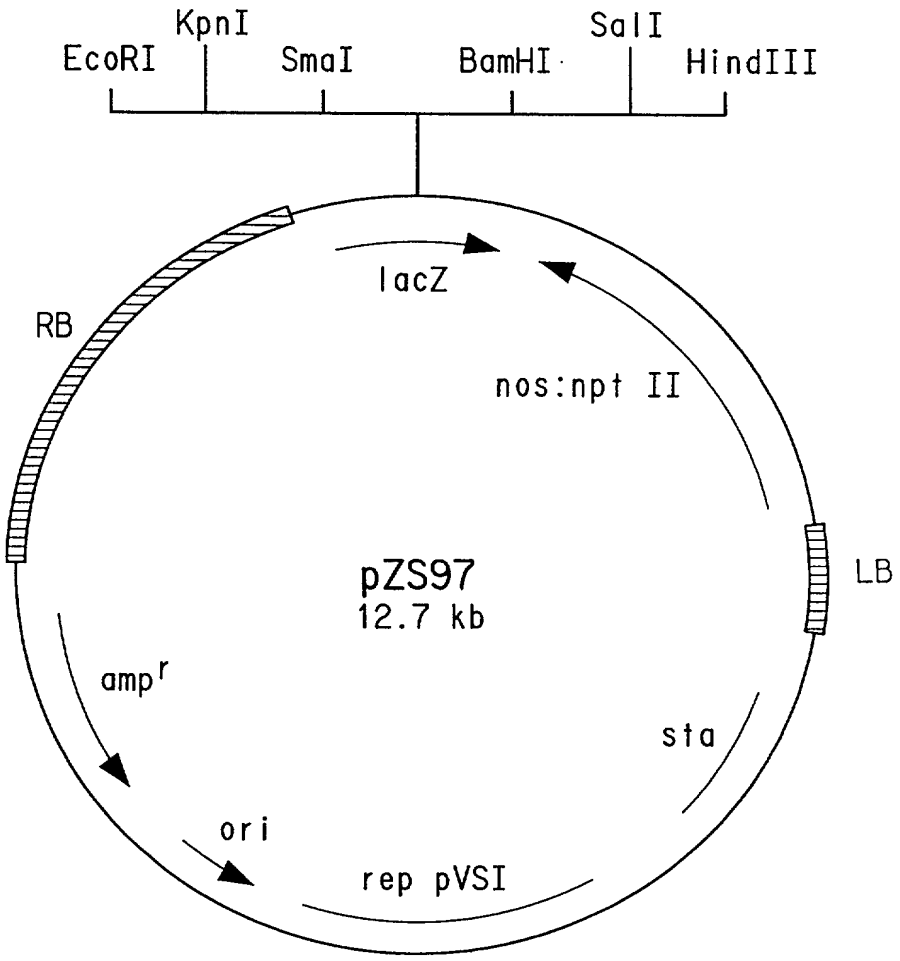
rep pVSI
ori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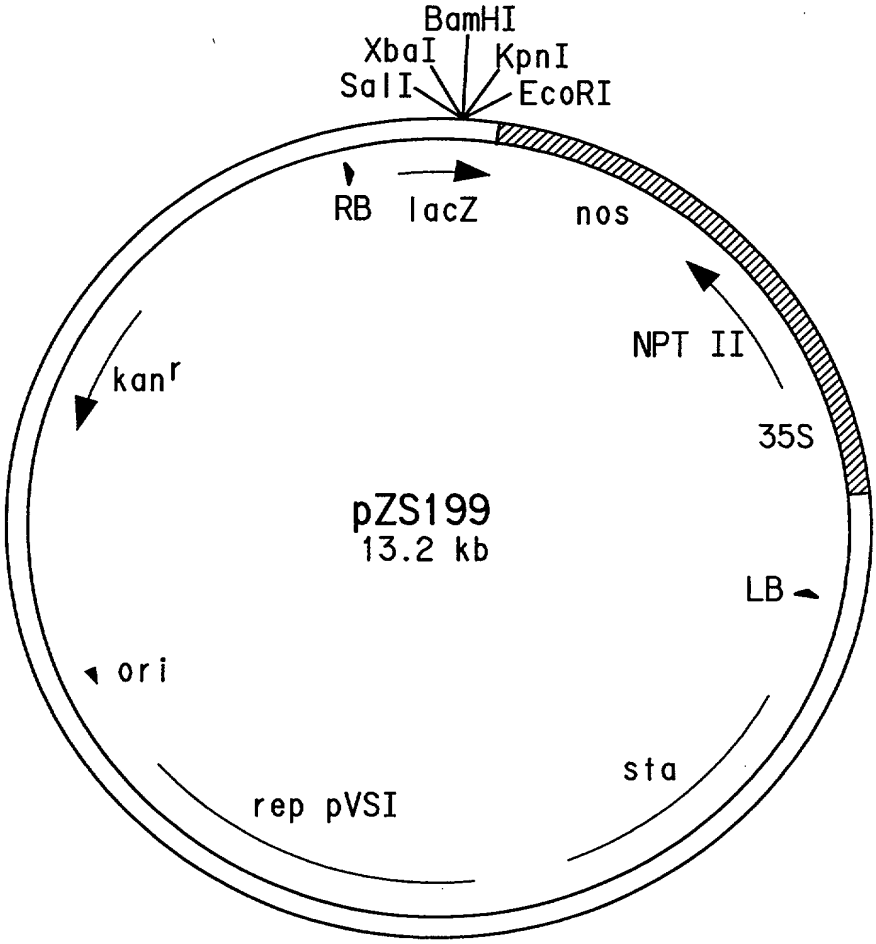
rep pVSI

FIG.6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sta
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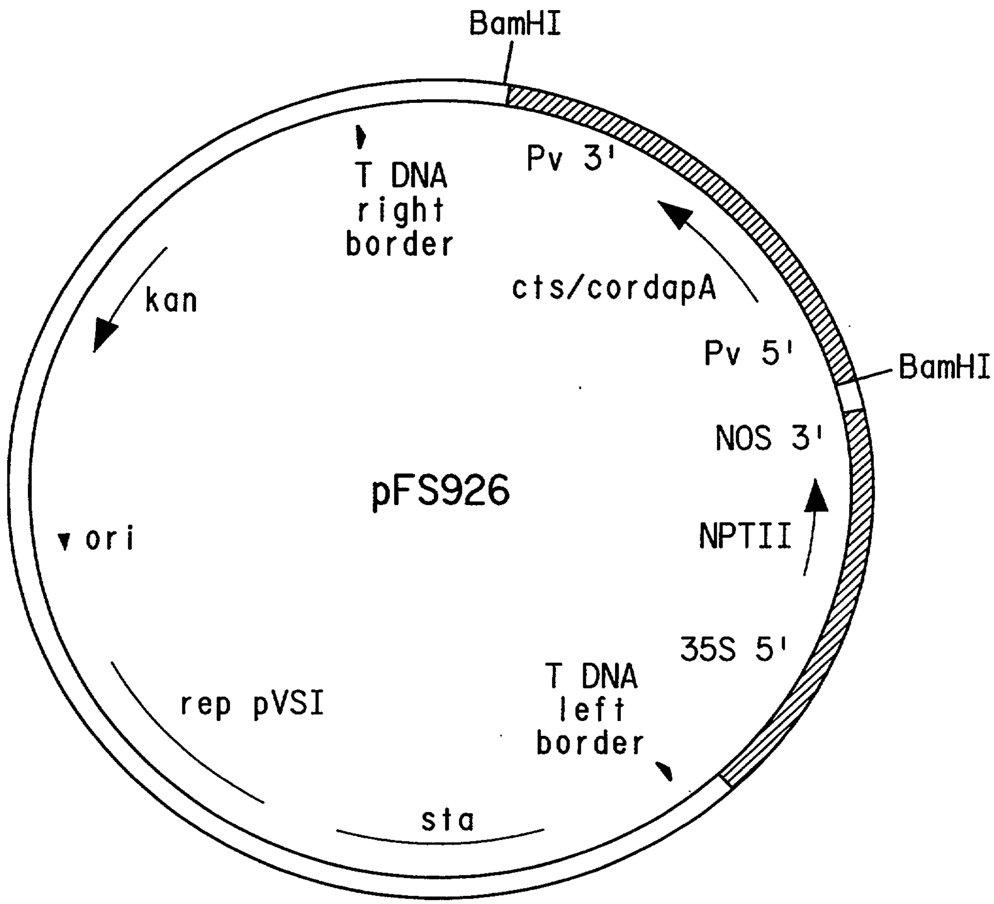
FIG.7A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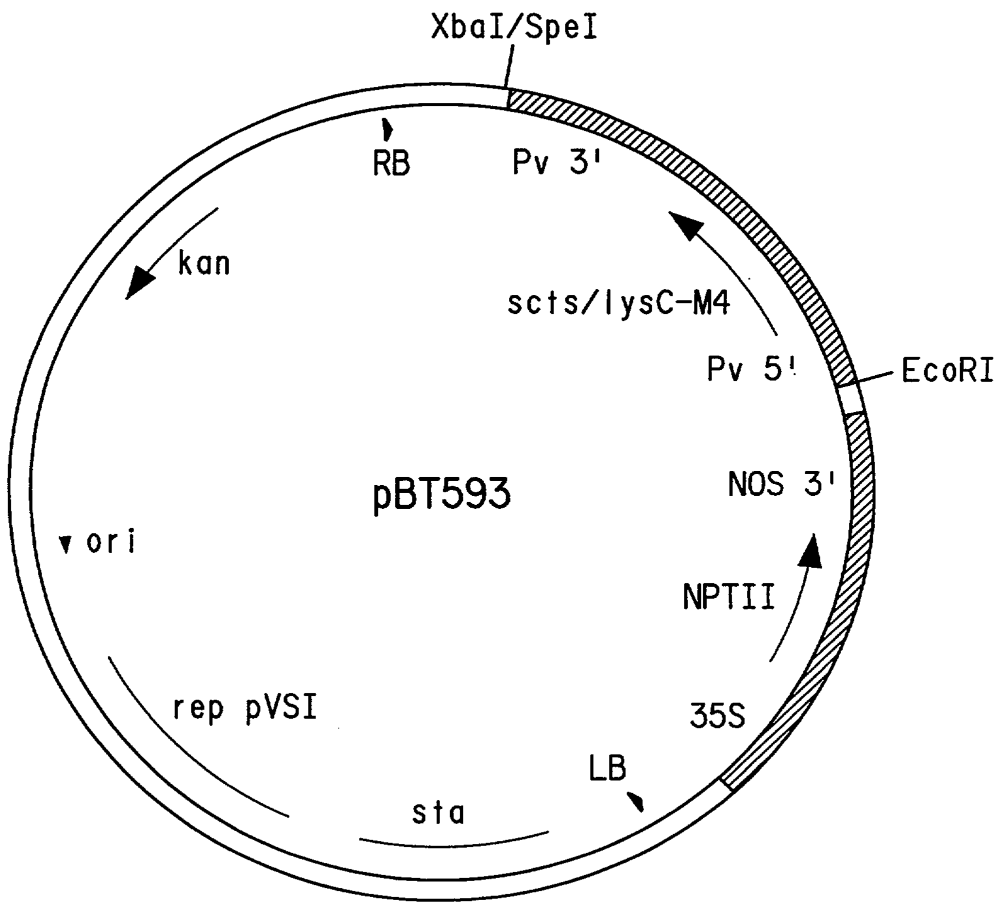
right
border
Pv 3'
cts/cordapA
kan
Pv 5'
BamHI
NOS 3'
pFS926
ori
NPTII
35S 5'
T DNA
left
border
rep pVSI
sta

FIG.7B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RB
Pv 3'
kan
scts/lysC-M4
Pv 5'
EcoRI
pBT593
NOS 3'
ori
NPTII
rep pVSI
35S
LB
sta

FIG.7C

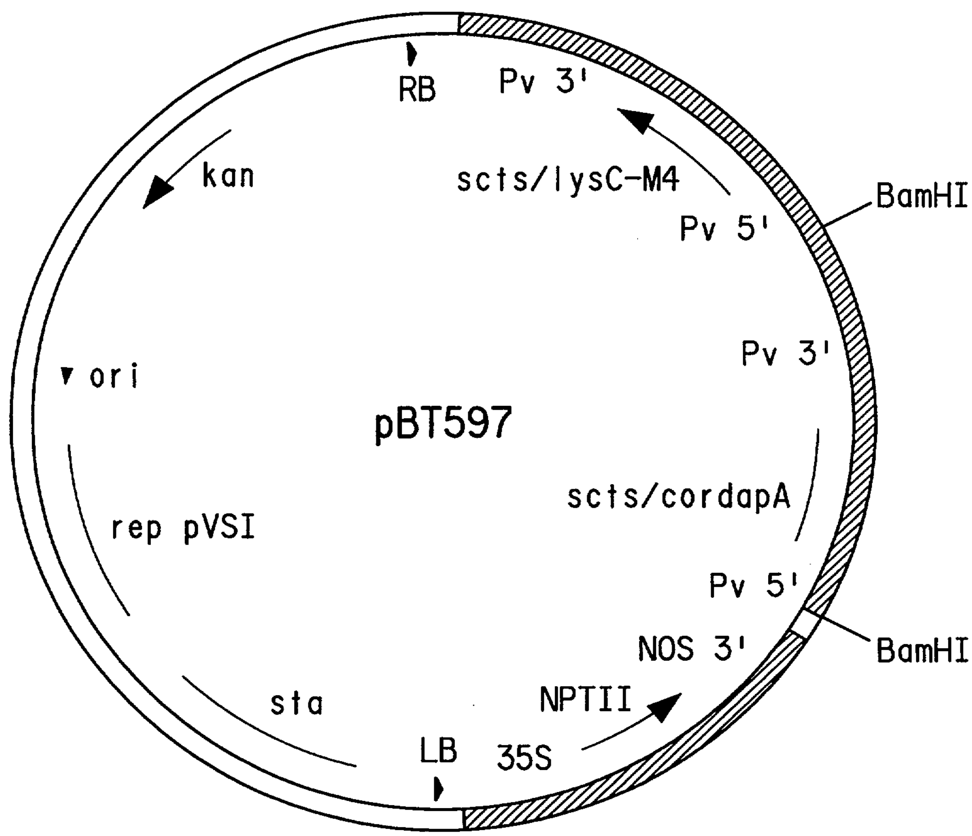


FIG.7D

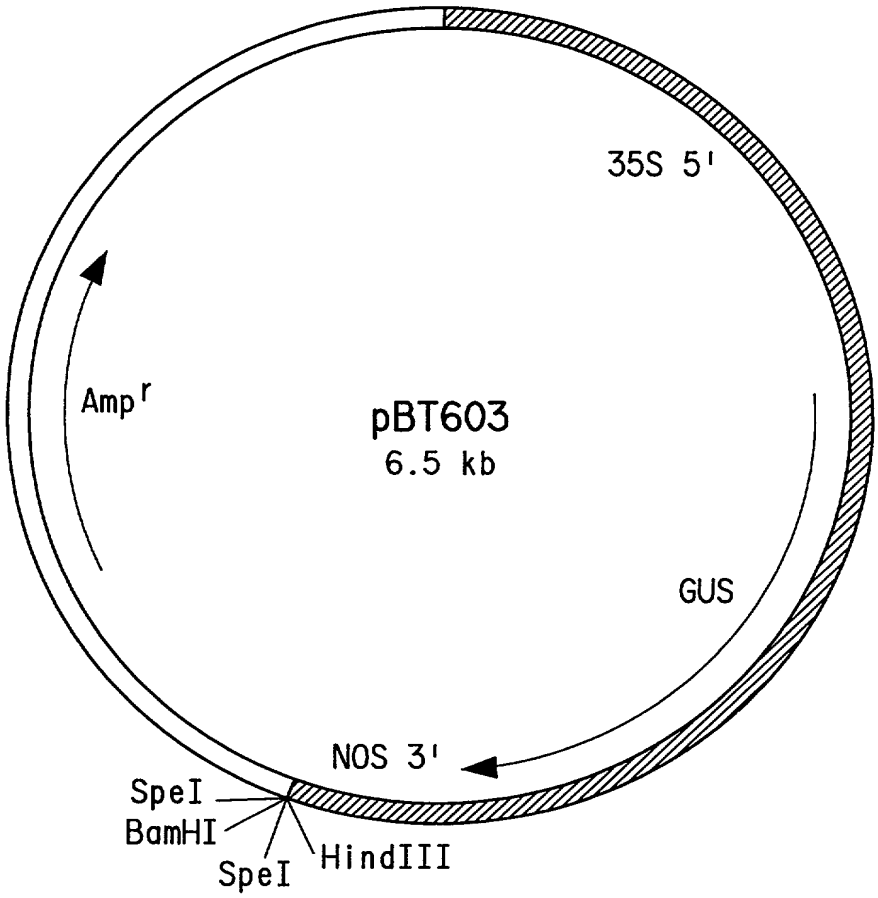
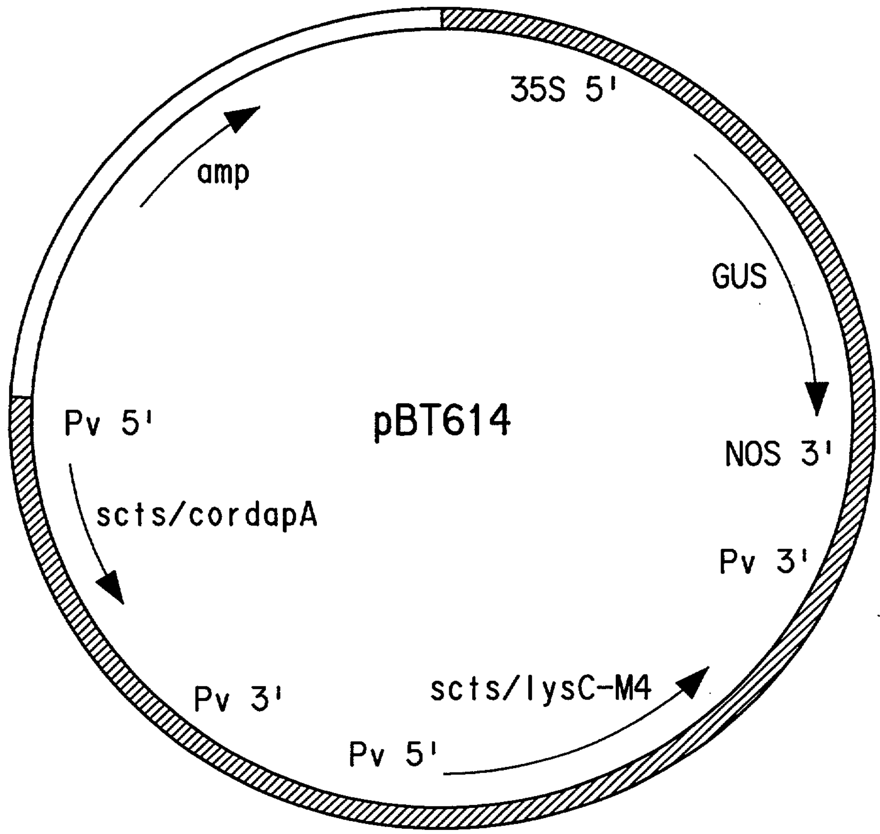


FIG.8A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amp
GUS
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NOS 3'
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Pv 3'
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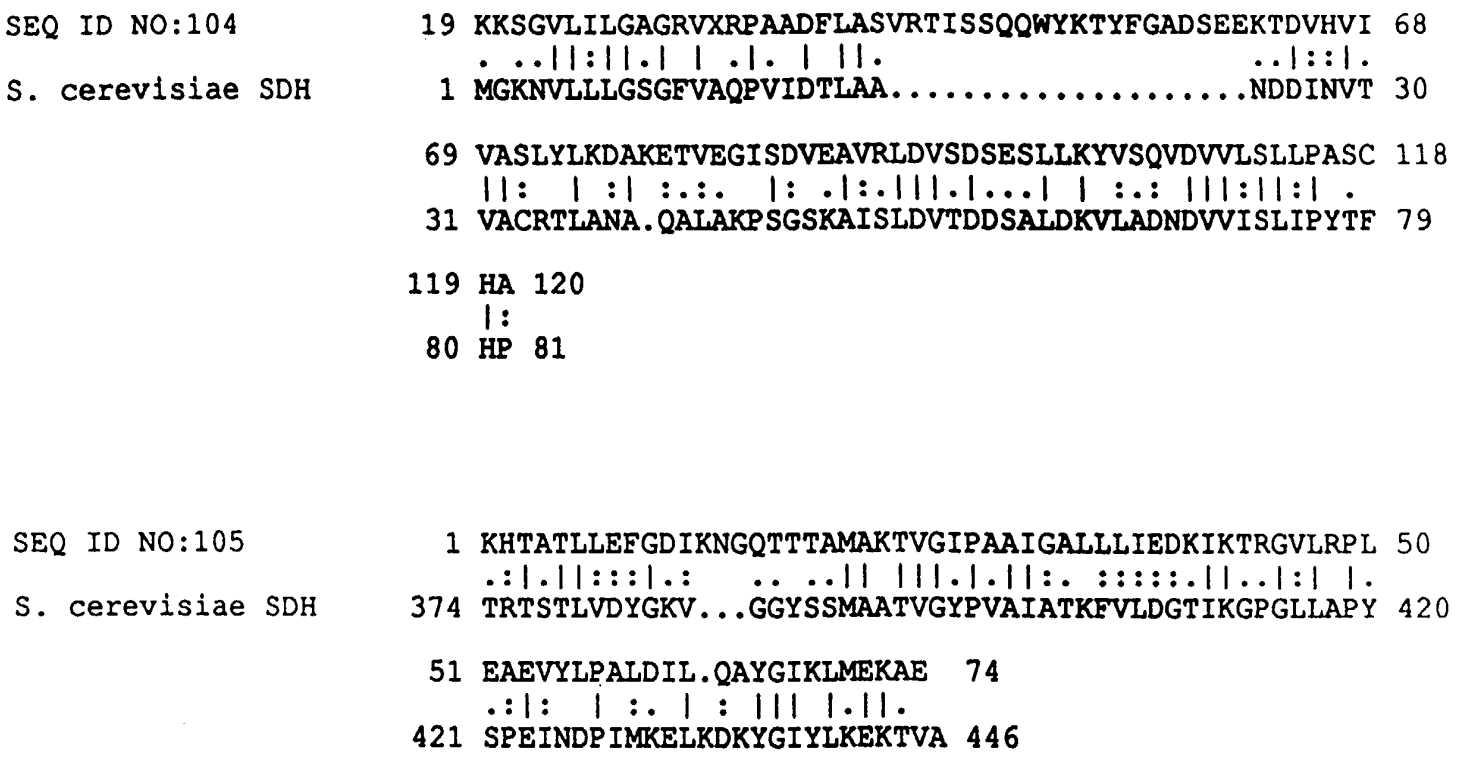
scts/lysC-M4
Pv 5'

FIG.8B

FIG.9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S. cerevisiae SDH
1 MGKNVLLLGSGFVAQPVIDTLAA.....NDDINVT 30
69 VASLYLKDAKETVEGISDVEAVRLDVSDSESLLKYVSQVDVVLSLLPASC 118
31 VACRTLANA.QALAKPSGSKAISLDVTDDSALDKVLADNDVVISLIPYTF 79
119 HA 120
80 HP 81
SEQ ID NO:105
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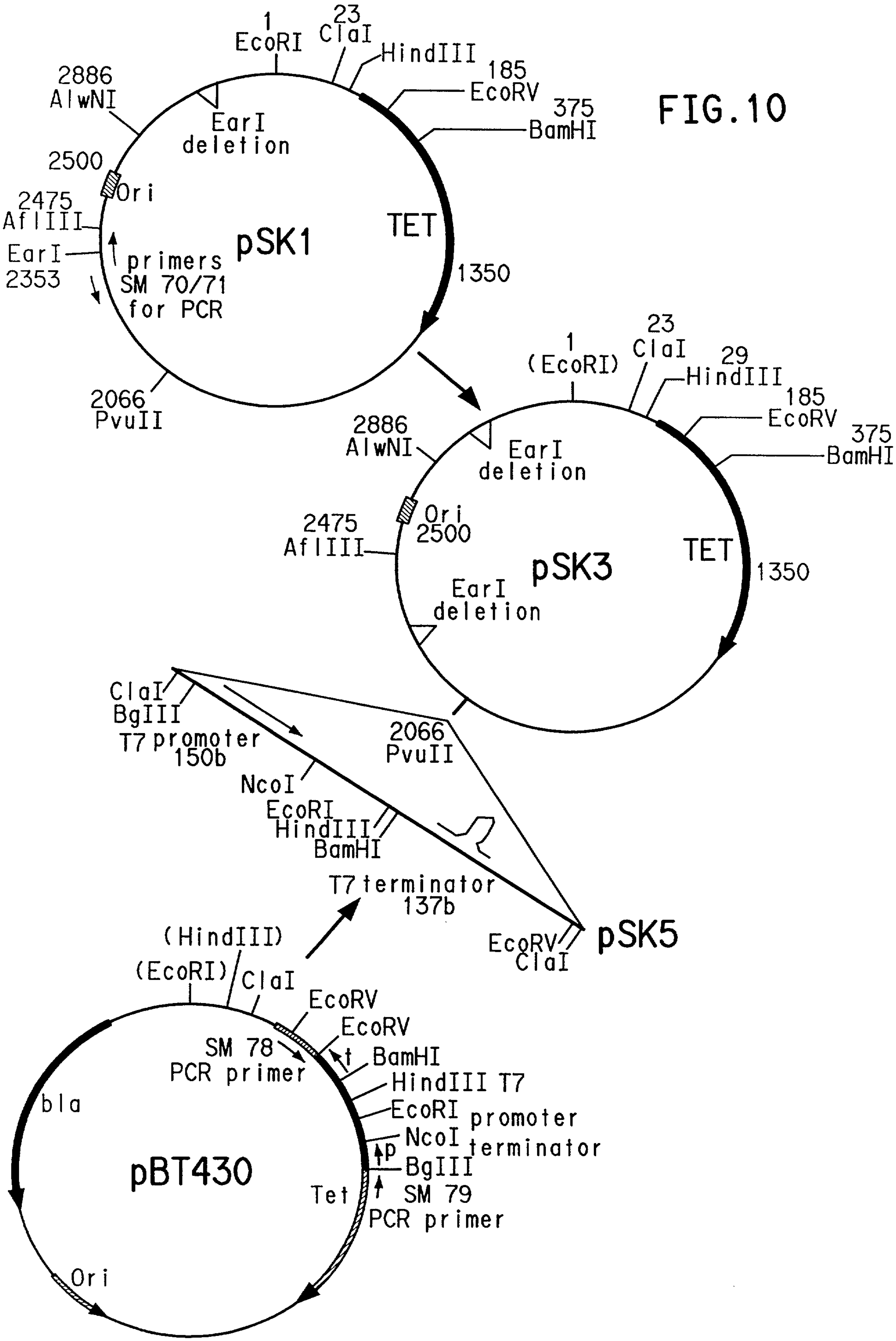
FIG.10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HindIII
185
EcoRV
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AlwNI
EarI
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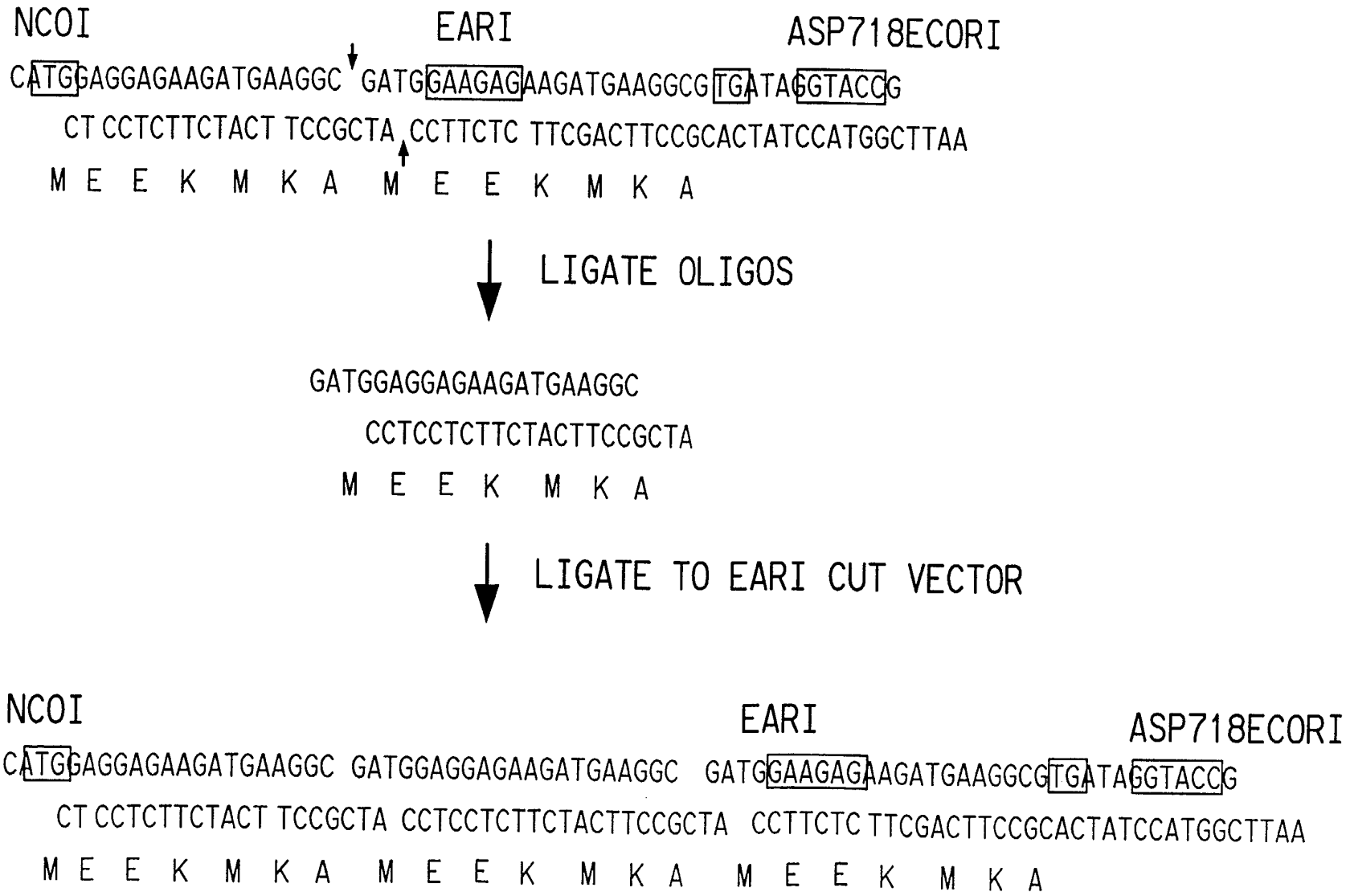
2475
AflIII
EarI
2353
primers
SM 70/71
for PCR
pSK1
TET
1350
2066
PvuII
1
(EcoRI)
23
ClaI
29
HindIII
185
EcoRV
375
BamHI
2886
AlwNI
EarI
deletion
Ori
2500
2475
AflIII
pSK3
TET
1350
EarI
deletion
ClaI
BglII
T7 promoter
150b
NcoI
EcoRI
HindIII
BamHI
2066
PvuII
T7 terminator
137b
EcoRV
ClaI
pSK5
(HindIII)
(EcoRI)
ClaI
EcoRV
EcoRV
SM 78
PCR primer
t
BamHI
HindIII T7
EcoRI promoter
NcoI terminator
p
BglII
SM 79
PCR primer
bla
pBT430
Tet
Ori

FIG.11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EARI
ASP718ECORI
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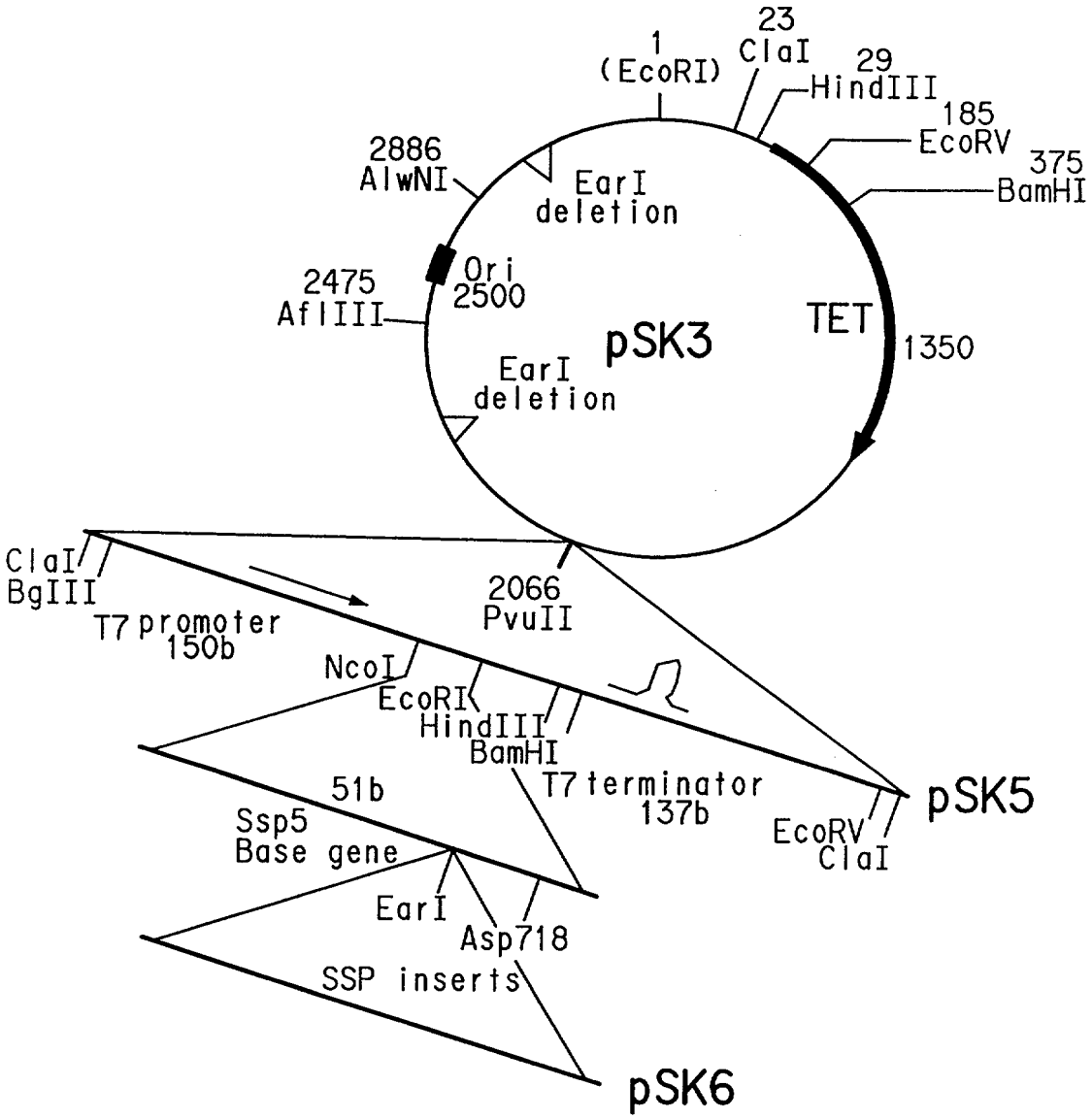
M E E K M K A M E E K M K A
LIGATE OLIGOS
GATGGAGGAGAAGATGAAGGC
CCTCCTCTTCTACTTCCGCTA
M E E K M K A
LIGATE TO EARI CUT VECTOR
NCOI
EARI
ASP718ECORI
CATGGAGGAGAAGATGAAGGC GATGGAGGAGAAGATGAAGGC GATGGAAGAGAAGATGAAGGCGTGATAGGTACCG
CT CCTCTTCTACT TCCGCTA CCTCCTCTTCTACTTCCGCTA CCTTCTC TTCGACTTCCGCACTATCCATGGCTTAA
M E E K M K A M E E K M K A M E E K M K A

FIG.12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185
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deletion
2475
AflIII
Ori
2500
pSK3
TET
1350
EarI
deletion
ClaI
BglII
T7 promoter
150b
2066
PvuII
NcoI
EcoRI
HindIII
BamHI
T7 terminator
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Ssp5
Base gene
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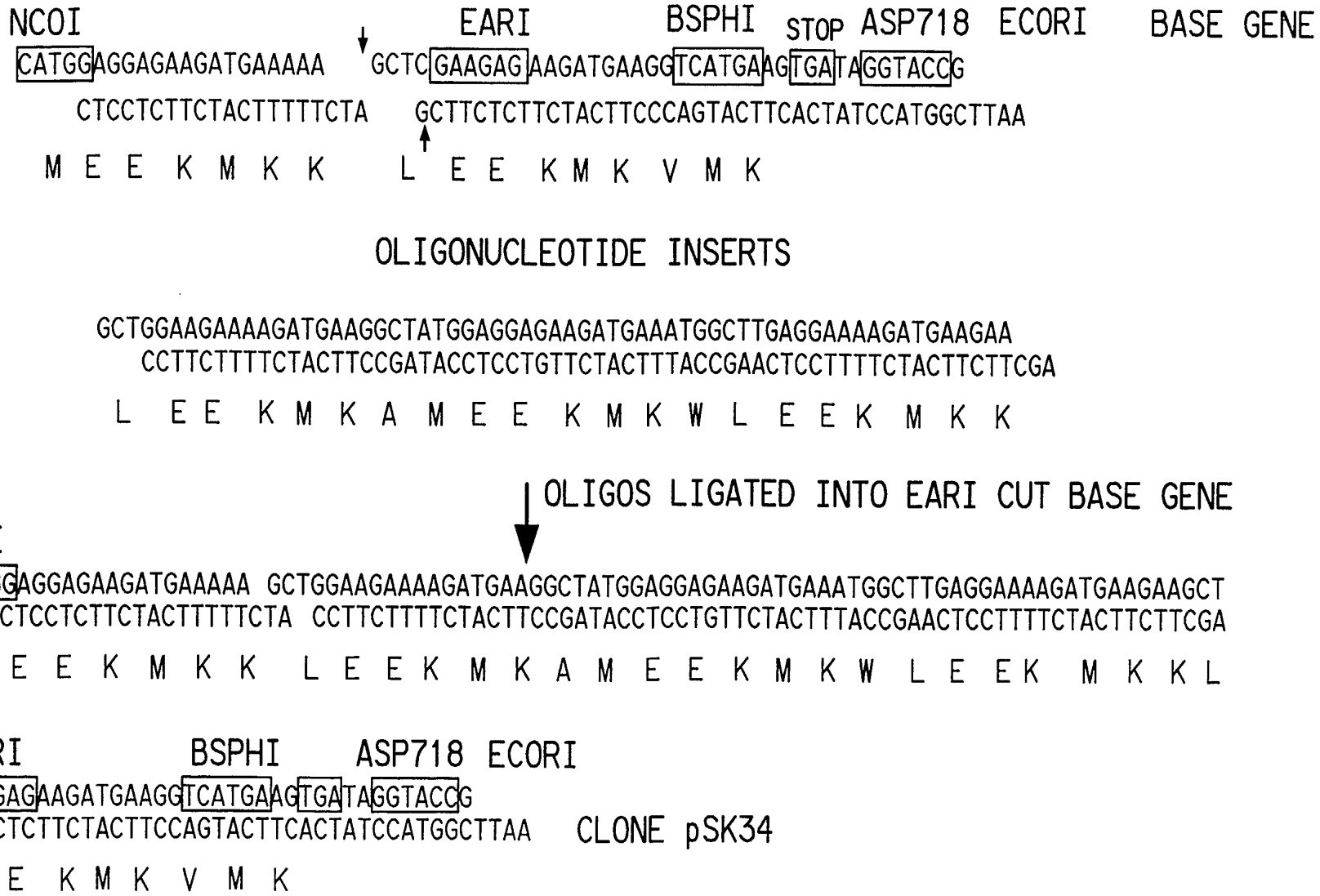
ClaI
EarI
Asp718
SSP inserts
pSK6

FIG.13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BSPHI
STOP
ASP718
ECORI
BASE GENE
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GCTTCTCTTCTACTTCCCAGTACTTCACTATCCATGGCTTAA
M E E K M K K
L E E K M K V M K
OLIGONUCLEOTIDE INSERTS
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CCTTCTTTTCTACTTCCGATACCTCCTGTTCTACTTTACCGAACTCCTTTTCTACTTCTTCGA
L E E K M K A M E E K M K W L E E K M K K
OLIGOS LIGATED INTO EARI CUT BASE GENE
NCOI
CATGGAGGAGAAGATGAAAAA GCTGGAAGAAAAGATGAAGGCTATGGAGGAGAAGATGAAATGGCTTGAGGAAAAGATGAAGAAGCT
CTCCTCTTCTACTTTTTCTA CCTTCTTTTCTACTTCCGATACCTCCTGTTCTACTTTACCGAACTCCTTTTCTACTTCTTCGA
M E E K M K K L E E K M K A M E E K M K W L E E K M K K L
EARI
BSPHI
ASP718
ECORI
CGAAGAGAAGATGAAGGTCATGAAGTGATAGGTACCG
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CLONE pSK34
E E K M K V M K

FIG. 14a

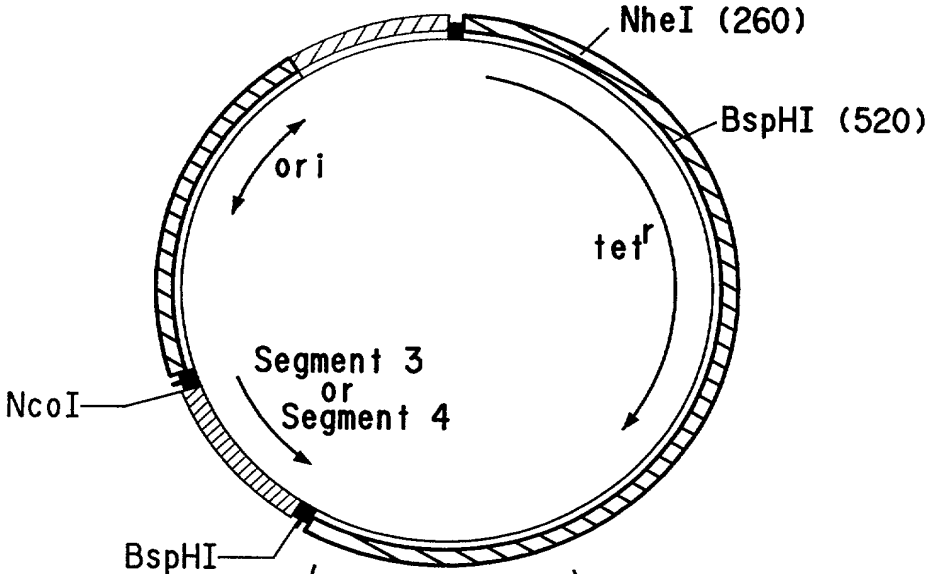


FIG. 14b

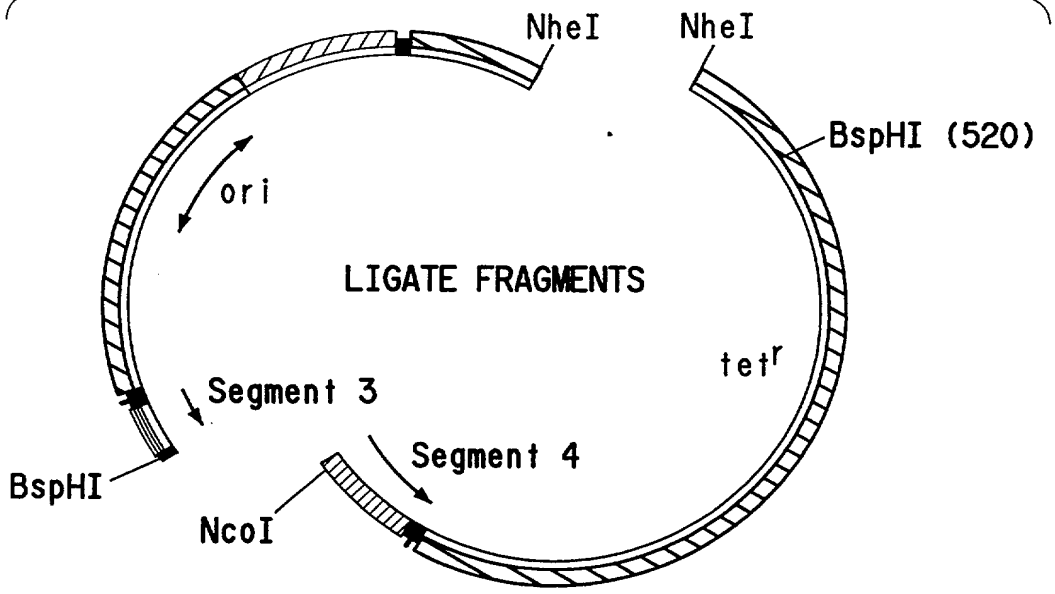
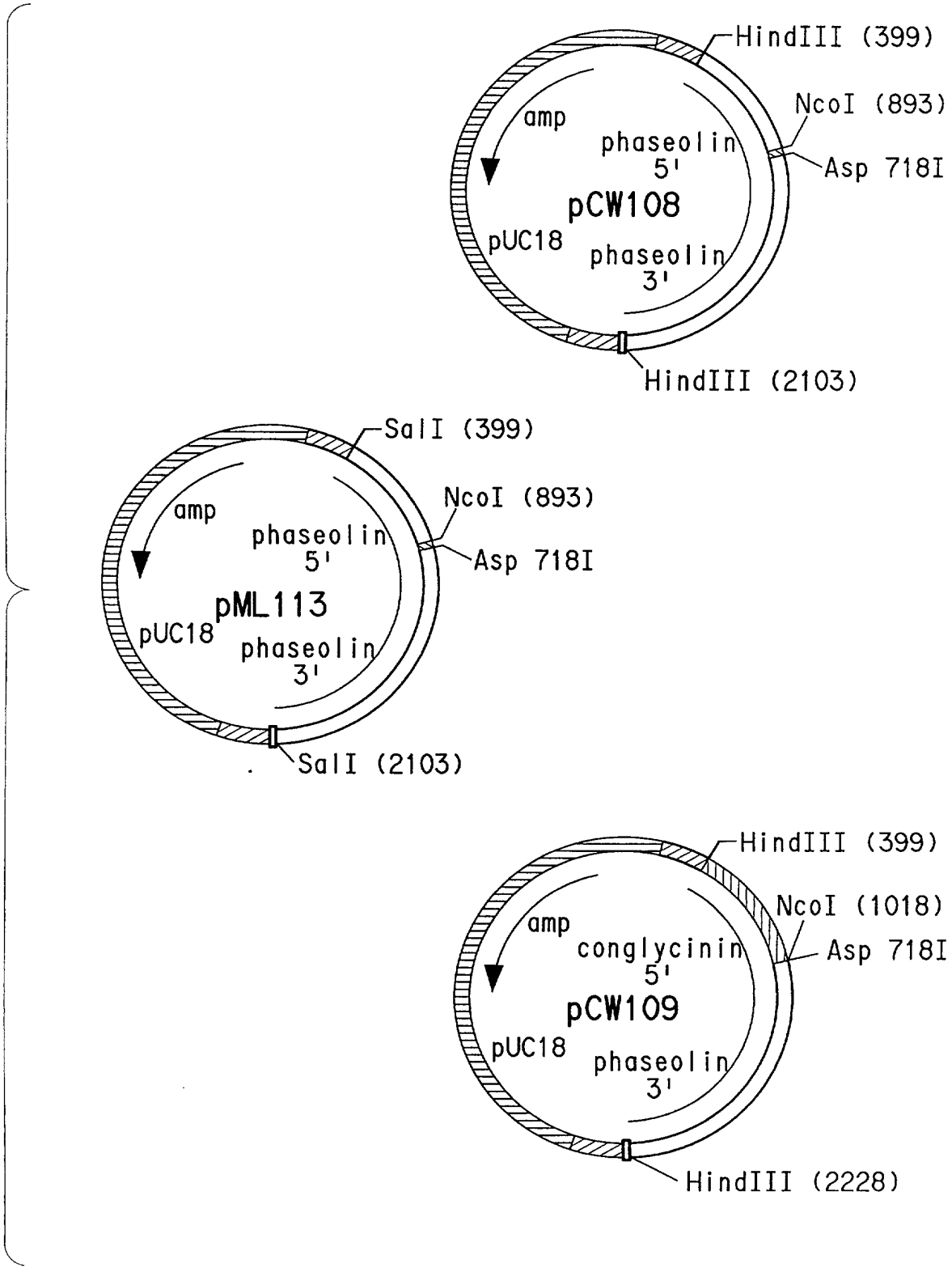


FIG.15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NcoI (893)
Asp 718I
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pCW108
pUC18
phaseolin 3'
HindIII (2103)
SalI (399)
NcoI (893)
Asp 718I
amp
phaseolin 5'
pML113
pUC18
phaseolin 3'
SalI (2103)
HindIII (399)
NcoI (1018)
Asp 718I
amp
conglycinin 5'
pCW109
pUC18
phaseolin 3'
HindIII (2228)

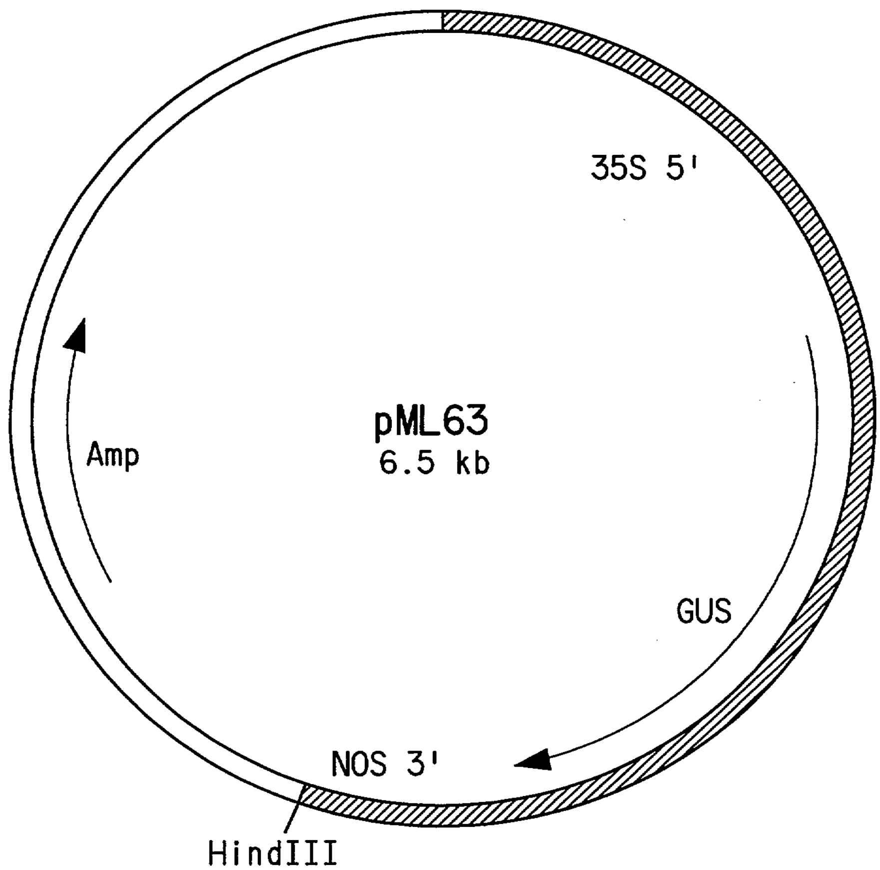

FIG.16

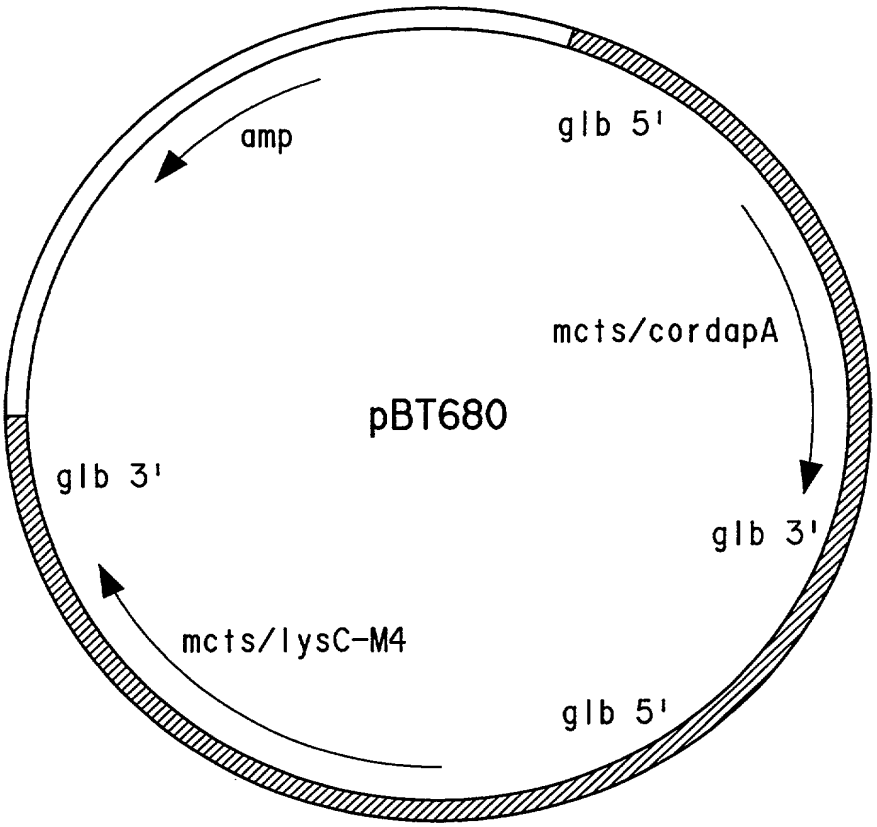

FIG.17

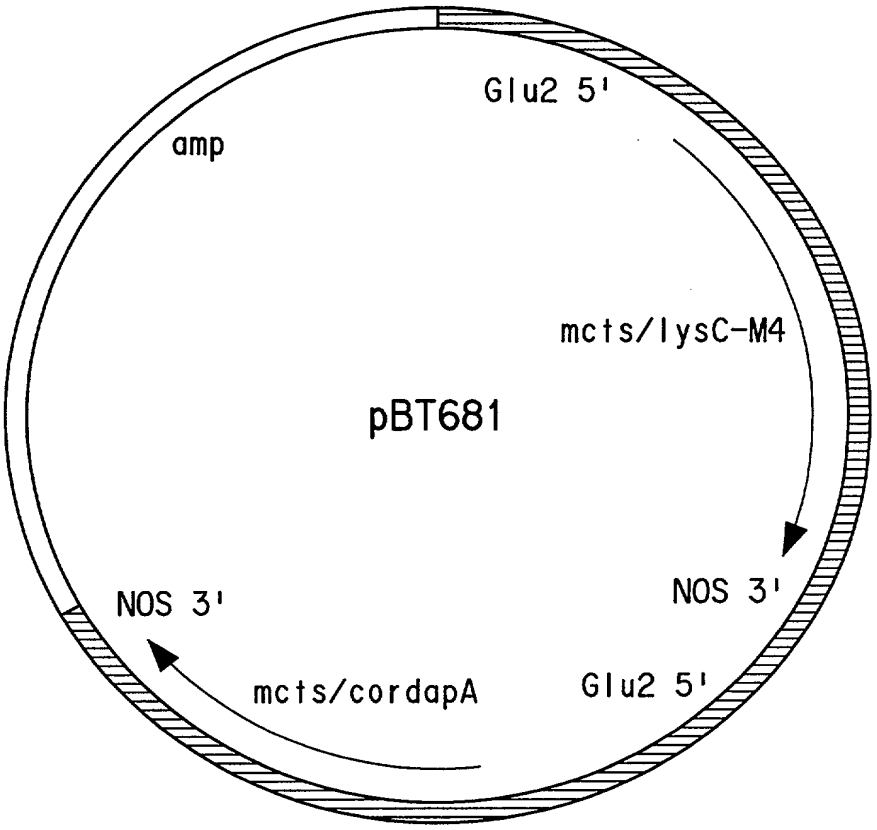

FIG.18

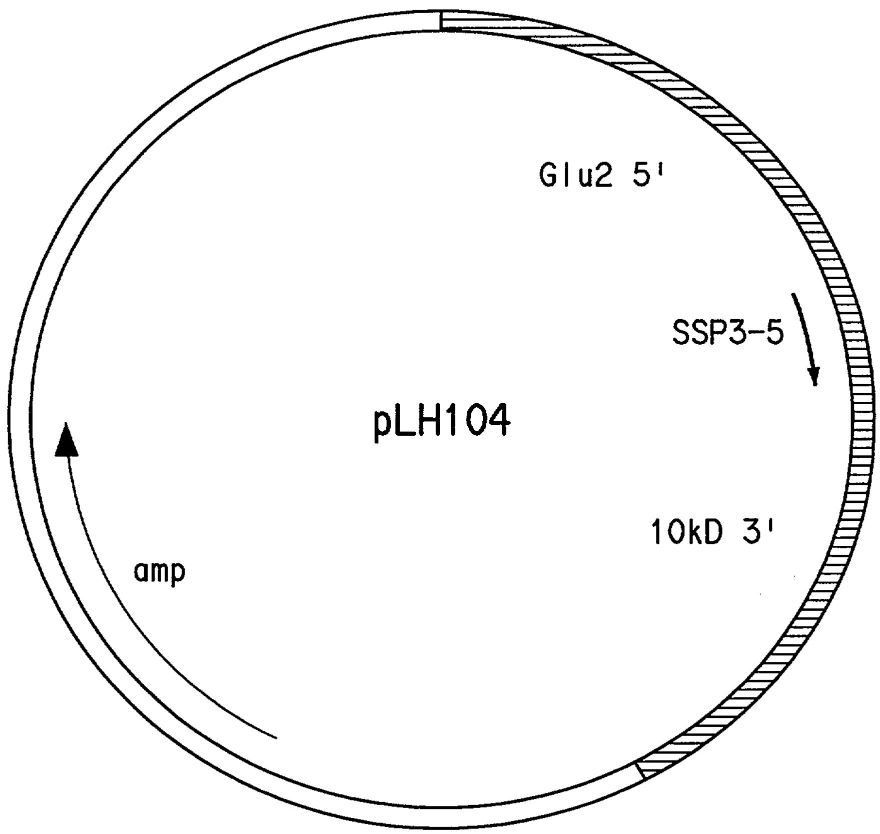

FIG.19

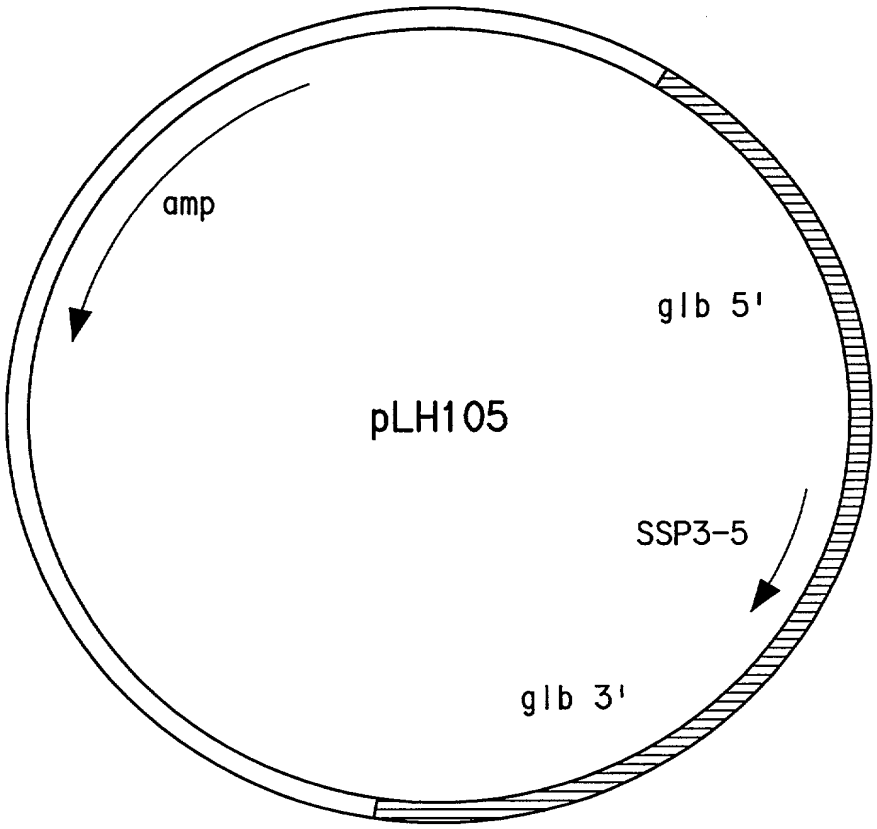

FIG.20

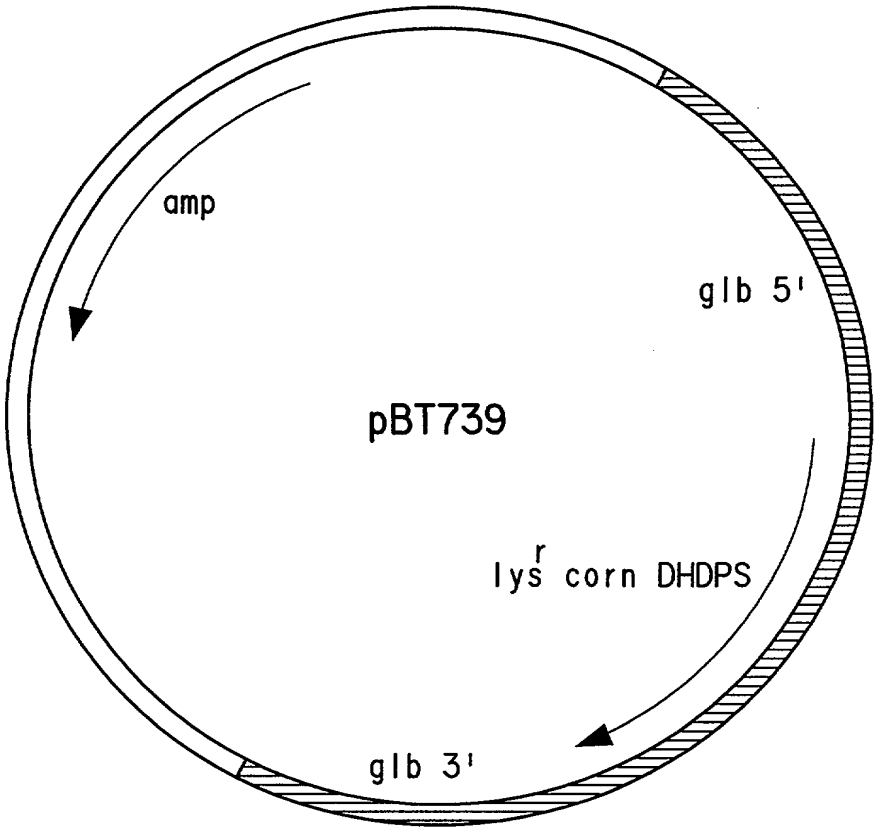

FIG.21

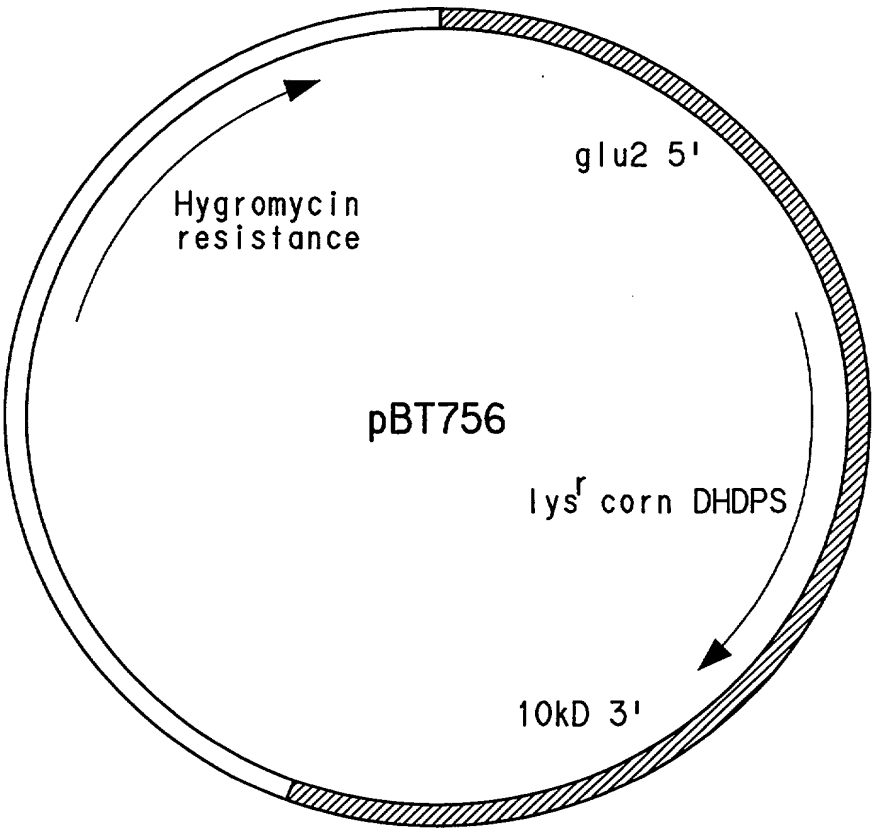

FIG.22

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